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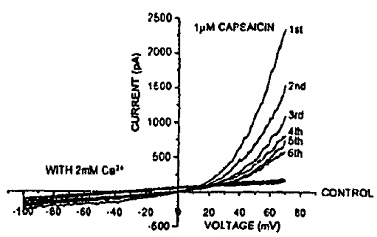
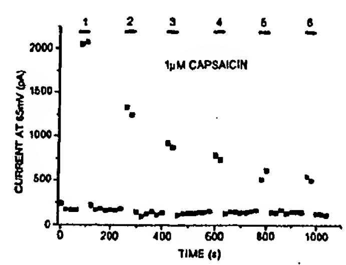
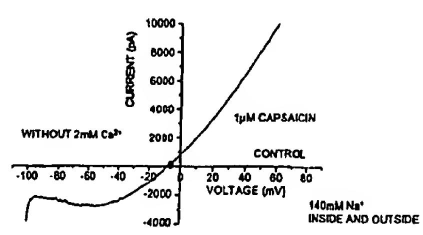
(54) Title: HUMAN VANILLOID RECEPTORS AND THEIR USES

(57) Abstract

The invention provides novel human vanilloid receptor (hVR) proteins, in particular hVR1 and hVR3, nucleotide sequences encoding for the novel hVR proteins, and hVR proteins for use in a method for screening for agents useful in the treatment or prophylaxis of disorders which are responsive to modulation of hVr activity in a human patient. The invention also provides expression vectors comprising said nucleotide sequences, stable cell lines comprising said expression vectors, antibodies specific for the novel hVR proteins, methods for the identification of compounds which exhibit hVR modulating activity, compounds identifiable and identified by such methods, and methods of treatment or prophylaxis of disorders which are responsive to modulation of hVR activity in a human patient.







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HUMAN VANILLOID RECEPTORS AND THEIR USES

Field of the Invention

The present invention relates to human vanilloid receptor (hVR) proteins and to related nucleotide sequences, expression vectors, cell lines, antibodies screening methods, compounds, methods of production and methods of treatment, as well as other related aspects.

Background of the Invention

Capsaicin, the irritant in hot peppers and a member of the vanilloid family activates a sub-group of sensory neurons: the nociceptors. These neurons transmit nociceptive and thermoceptive pain information back to pain-processing centres in the central nervous system such as the spinal cord and the brain. They are also sites for the release of pro-inflammatory mediators in the periphery (1). Nociceptors show heterogeneity in their sensitivity to capsaicin. Excitation and prolonged exposure of these neurons to capsaicin is followed by a refractory state known as desensitisation (2) when they become insensitive to capsaicin and other noxious stimuli (3). The long-term response to insensitivity could be explained by death of the nociceptors or destruction of its peripheral terminals (4). Because of the desensitisation phenomenon, capsaicin has been used therapeutically for decades as an analgesic agent for the treatment of pain in a range of disorders (5).

It has been speculated that the endogenous target for capsaicin plays an important function in the detection of painful stimuli. It has been shown by electrophysiological and biochemical studies that capsaicin induces a flux of cations in dorsal root ganglion (DRG) neurons (6,7). Because other vanilloid derivatives show responses in a dose dependent manner (8,9) a receptor is the most likely candidate to explain the mechanism. Therefore, based on indirect evidence it has been anticipated that these actions of capsaicin (excitation / desensitisation) are mediated by a specific membrane-bound receptor named vanilloid receptor (10).

Evidence for the existence of a vanilloid receptor came from binding experiments with resiniferatoxin (RTX), a capsaicin analog (11), and a competitive antagonist

of capsaicin, capsazepine (12). Vanilloid receptors have been visualised by using ([^{3}H]-RTX) autoradiography in dorsal root ganglia (DRG) and spinal cord of different species including man (13,14).

Recently, a rat vanilloid receptor termed VR1 has been identified using an expression-cloning strategy to isolate the complementary DNA (cDNA) encoding the corresponding protein from a rat DRG cDNA library (15). The cDNA clone was completely sequenced. The rat VR1 cDNA has an open reading frame of 2,514 nucleotides and encodes for a protein of 838 amino acids with a predicted relative molecular mass of 95,000. Analysis of the amino acid sequence identified 6 potential transmembrane regions with a short hydrophobic stretch between the transmembrane regions 5 and 6. The N-terminus (amino terminal) contains three ankyrin repeat domains. No motifs have been identified at the C-terminus (carboxy terminal).

It has been noted that rat VR1 transfected cells exhibit an increase in calcium levels after heat treatment and it has been suggested that *in vivo* VR1 and vanilloid receptors are involved in detection of noxious heat (but not innocuous heat). It has also been proposed that protons could act as modulators of the vanilloid receptors (16, 17, 18).

While it has been recognised that the rat capsaicin receptor, VR1, is a member of the family of non-selective ion channels that are gated by ligands and that it is involved in pain sensation, the natural ligand of VR1 remains unknown. It is therefore suggested that human vanilloid receptor sub-types may provide targets for the development of novel analgesic agents (agonists and antagonists) and agents which may interact with other disorders.

Accordingly, it is an object of the present invention to locate and characterise human vanilloid receptors. Other objects of the present invention will become apparent from the following detailed description thereof.

Summary of the Invention

According to one embodiment of the present invention there is provided an isolated human vanilloid receptor (hVR) protein or a variant thereof. Preferably

the hVR protein is an hVR1 or hVR3 protein or a variant thereof. In a particularly preferred aspect of the invention the hVR protein has an amino acid sequence as shown in figure 3 or in figure 18.

According to another aspect of the invention, there is provided a human vanilliod receptor (hVR) protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, for use in a method of screening for agents useful in the treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as diabetic neuropathy, incontinence and interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a nucleotide sequence encoding a human vanilloid receptor (hVR) protein or a variant thereof as hereinbefore described, or a nucleotide sequence that is complementary thereto. Preferably the nucleotide sequence encodes an hVR1, hVR3 protein or variant thereof or a nucleotide sequence which is complementary thereto. Particularly preferably the nucleotide sequence is as shown in figure 2 and figure 17.

According to another aspect of the invention there is provided an expression vector comprising a nucleic acid sequence as referred to above which is capable of expressing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof. Preferably the expression vector is as displayed in figure 6 or figure 20.

According to another aspect of the invention there is provided a stable cell line comprising an expression vector as referred to above which is capable of expressing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof. The stable cell line is preferably a

modified mammalian cell line, preferably HEK293, CHO, COS, HeLa or BHK although transient expression may be preferred in *Xenopus* oocytes.

According to another aspect of the invention there is provided an antibody specific for an hVR protein as hereinbefore described or a variant thereof, preferably specific for hVR1 or hVR3 or a variant thereof.

According to another aspect of the invention there is provided a method for identification of a compound which exhibits hVR modulating activity, comprising contacting an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, with a test compound and detecting modulating activity or inactivity.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, for use in therapy.

According to another aspect of the invention there is provided the use of a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR,

preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identifiable by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine.

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According to another aspect of the invention there is provided the use of a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine, in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic

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pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a compound identified by the method referred to above.

According to another aspect of the invention there is provided a compound identified by the method referred to above, for use in therapy.

According to another aspect of the invention there is provided the use of a compound identified by the method referred to above in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic

obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identified by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a method of producing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, under conditions suitable for obtaining expression of the hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof.

Brief Description of the figures

Figure 1 is an alignment of hVR1 *in silico* derived clusters with rat VR1.

Figure 2 displays the human VR1 nucleotide sequence including the 5'UTR (nt –773 to nt 0), coding region (nt 1 to 2517) and 3'UTR (nt 2518 to nt 3560).

Figure 3 illustrates the nucleotide and encoded amino acid sequence of the human VR1sequence.

Figure 4 depicts the amino acid sequence of the hVR1 gene, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed). The predicted phosphorylation sites are underlined.

Figure 5 is a comparison of the amino acid sequences of the rat (rVR1) and human (hVR1) vanilloid receptors.

Figure 6 illustrates constructs pBluescriptSK(+) (A) and pCIN5-new (B) with the full length hVR1 gene cloned via NotI and EcoRI restriction sites.

Figure 7 shows a Slot Blot hybridisation with hVR1 probe with positive labelling of both rat and human DRG mRNA.

Figure 8 displays a Western blot probed with anti-VR1 antibodies with the arrow indicating the VR1 specific protein.

Figure 9 shows localisation of VR1 in rat DRG tissue sections, the arrow points to VR1 expressing small diameter (<25μn) neurone cell bodies.

Figure 10 depicts the *in situ* localisation of VR1 in human DRG sections (A) and human skin (B).

Figure 11 illustrates the functional response to capsaicin and blockade by capsazepine (CPZ) (A) with the current voltage relationship plotted in (B) on human VR-1 channels, transiently expressed in HEK293T cells.

Figure 12 shows capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium (A), maximum current (65mV) against time (B) and current voltage relationship in the absense of Ca^{2+} (C).

Figure 13 shows the influx of calcium into transiently transfected HEK293T cells over a time course in the presence of agonist capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 14 illustrates a graphical presentation the results shown in figure 13 examining the response of hVR1 transfected HEK293T cells over time before and after exposure to agonists: capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 15 displays the proposed assay strategy to carry out drug screening.

Figure 16 displays an alignment of *in silico* derived hVR3 specific clusters with rat VR1.

Figure 17 depicts the hVR3 nucleotide sequence including the 5' UTR (nt –686 to nt 0) Coding region (nt1 to nt 2889), 3'UTR (nt 2890 to nt 3418).

Figure 18 shows the nucleotide and amino acid sequence of hVR3.

Figure 19 is of the amino acid sequence of hVR3, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Figure 20 displays constructs pBluescriptSK(+) (A) and pCDNA3.1 (+) (B) with the full length hVR3 gene cloned via NotI and XhoI restriction sites.

Figure 21 illustrates a multiple comparison of the amino acid sequences of the rat VR1 and the human vanilloid receptors: hVR1, hVRL-1 and hVR3.

Figure 22 Northern Blot hybridisation with hVR3 probe with strong signals detected in trachea (A), prostate (B), placenta, kidney and pancreas (C).

Detailed Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

As referred to above, the present invention relates to isolated human vanilloid receptor (hVR) proteins, and in particular to the human vanilloid receptors which will be termed respectively human vanilloid receptors 1 and 3 (hVR1, and hVR3), sequence information for which is provided in figures 2 (hVR1) and 17 (hVR3). In the context of this invention the term "isolated" is intended to convey that the receptor protein is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the receptor protein being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the receptor protein is in a state as found in nature.

Routine methods, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the receptor proteins according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. *et al.* (28), the disclosure of which is included herein in its entirety by way of reference.

By the term "variant" what is meant throughout the specification and claims is that other peptides or proteins which retain the same essential character of the human vanilloid receptor proteins for which sequence information is provided, are also intended to be included within the scope of the invention. For example,

other peptides or proteins with greater than about 80%, preferably at least 90% and particularly preferably at least 95% homology with the sequences provided are considered as variants of the receptor proteins. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the biological functionality of a human vanilloid receptor. This biological functionality can of course be assessed by conducting binding studies with known vanilloid modulators such as capsaicin, capsazepine (12) and resiniferatoxin (11).

Human VR1 is preferentially expressed in human dorsal root ganglia (DRG) and relative to hVR3 has the highest sequence homology with the rat VR1. Therefore, hVR1 is likely to be the human orthologue to rat VR1. hVR3 is less similar to rat VR1 and is expressed in a wider range of tissues. Nucleotide sequence analysis of hVR1 reveals a 2517bp open reading frame which encodes an 839 amino acid protein (see figures 2, 3 and 4). This deduced hVR1 protein sequence is 86 % identical to the rat VR1 (15) and shares many of its characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains. Similarly hVR3 has an open reading frame of 2889bp open reading frame which encodes a 963 amino acid protein (see figures 17, 18 and 19). The deduced hVR3 protein is 46 % identical to rat VR1 and 44 % identical to hVR1 sharing many of VR1's characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains.

The invention also includes nucleotide sequences which encode for human vanilloid receptor proteins or variants thereof as well as nucleotide sequences which are complementary thereto. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. Preferably the proteins are hVR1, hVR3 or variants thereof. Such nucleotides can be isolated or synthesised according to methods well know in the art. See reference 28, the disclosure of which is included herein in its entirety by way of reference.

The present invention also includes expression vectors which comprise nucleotide sequences ncoding for the hVR, preferably hVR1 or hVR3, receptor

proteins or variants thereof. A further aspect of the invention relates to an expression vector comprising nucleotide sequences encoding for hVR1 or hVR3 receptor proteins or variants thereof. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Suitable vectors for use in practicing the present invention include pBluescript (Stratagene), pCR-Script (Stratagene), pCR2.1-TOPO (Invitrogen), pCRII-TOPO (Invitrogen), pCR-Blunt (Invitrogen), with vectors such as pCIN (32) (available from Clontech as pIRES-neo), pCDNA 3.1 (Invitrogen) or pCIneo (Promega) required for mammalian expression. Appropriate methods can be effected by following protocols described in many standard laboratory manuals (28, 29).

The invention also includes cell lines which have been modified to express the novel receptor. Such cell lines include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which have been modified by insertion of vectors encoding for the receptor proteins according to the invention include HEK293T cells and *Xenopus* oocytes. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of the inventive receptor. Representive examples of appropriate hosts include animal cells such as HEK293, CHO, COS, HeLa and BHK.

It is also possible for the receptors of the invention to be transiently expressed in a cell line or on a membrane, such as for example in a baculovirus expression system. Such systems, which are adapted to express the receptors according to the invention, are also included within the scope of the present invention.

In particular, the functional hVR protein may include hVR receptor proteins selected from hVR1 and hVR3 and thereof or even other hVR protein subtypes or splice variants which have not yet been identified.

According to another aspect, the present invention also relates to antibodies, preferably monoclonal antibodies, which have been raised by standard techniques and are specific for the receptor proteins or variants thereof according to the invention. Such antibodies could for example. be useful in purification; isolation or screening involving immuno precipitation techniques and may be used as tools to further ellucidate hVR, preferably hVR1 or hVR3, protein function, or indeed as therapeutic agents in their own right. Antibodies may also be raised against specific epitopes of the receptors according to the invention.

An important aspect of the present invention is the use of receptor proteins according to the invention in screening methods designed to identify compounds which act as receptor ligands and which may be useful to modulate receptor activity. In general terms, such screening methods will involve contacting the receptor protein concerned, preferably hVR1 or hVR3, with a test compound and then detecting modulation in the receptor activity, or indeed detecting receptor inactivity, which results. For further details on the screening strategy refer to figure 15. The present invention also includes within its scope those compounds which are identified as possessing useful hVR, preferably hVR1 or hVR3, modulation activity, by the screening methods referred to above. The screening methods comprehended by the invention are generally well known to persons skilled in the art. High throughput screens may include fluorescence based assays using the Fluorometric Imaging Plate Reader (FLIPR) with calcium sensitive dyes, and reporter gene assays using calcium sensitive photoproteins that emit light on the influx of calcium and can be detected using an Imaging system. Secondary screens may involve electrophysiological assays utilising patch clamp technology to identify small molecules, antibodies, peptides, proteins or other types of compounds that interact with hVR, preferably hVR1 or hVR3, to modulate activity. Tertiary screens may involve the study of modulators in well characterised rat and mouse models of pain. These models of pain include, but are not restricted to, intraplantar injection of inflammatory agents such as carageenan, formalin and complete freunds adjuvant (CFA). Models of neuropathic pain such as loose ligature of the sciatic nerve are also included. Other screens may involve the study of modulators in human volunteers subject to topically applied capsaicin.

Another aspect of the present invention is the use of compounds which have been identified by screening techniques referred to above in the treatment or prophylaxis of disorders which are responsive to modulation of hVR, preferably hVR1 or hVR3, receptor activity, in a human patient. By the term "modulation" what is meant is that there will be either agonism or antagonism at the receptor site which results from ligand binding of the compound at the receptor. By the term "modulation" what is meant is that there will be either agonism or antagonism at the receptor site which results from ligand binding of the compound at the receptor excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine. hVR , preferably hVR1 and hVR3, proteins have been implicated in disorders of the central nervous system (CNS), gastrointestinal (GI) tract, lungs and bladder and therefore modulation of hVR, preferably hVR1 or hVR3, receptor activity in these tissues will result in a positive therapeutic outcome in relation to such disorders. In particular, the compounds which will be identified using the screening techniques according to the invention will have utility for treatment and/or prophylaxis of disorders such as pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, IBS, respiratory disorders such as asthma and COPD, urological disorders including diabetic neuropathy, incontinence and interstitial cystitis, and inflammatory disorders. It is to be understood however, that the mention of such disorders is by way of example only, and is not intended to be limiting on the scope of the invention.

The compounds which are identified according to the screening methods outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art, and as fully described in Remmington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed, 1985, the disclosure of which is included herein in its entirety by way of reference.

The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

The present invention will be further explained, by way of examples, in the appended experimental section. Reference examples are provided.

Experimental details

Reference Example A: Identification of related human ESTs (Expressed Sequence Tags) (19) to the rat VR1 sequence by *in silico* analysis

The full-length rat VR1 amino acid sequence (15) was used as a query sequence using the tBlastn (20) alignment program to identify related human genes in the dbEST (21) and Incyte (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, California 94304, USA) databases. Several human ESTs were identified and those with similarities greater than 50% selected for further analysis. One of these ESTs was T12251 previously shown to have 68% amino-acid identity and 84% similarity over a region of 70 amino acids (15). Full-length cloning and functional characterisation of the gene represented by this cluster has been completed (30). This gene was denoted hVRL-1 and encoded a protein of 764 amino acid protein that was 48 % identical to the rat VR1 protein. All human ESTs from both databases were clustered to identify overlapping identical ESTs belonging to the same transcript. The GCG package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin) and a program developed in house termed ESTBlast (22) were used to build up these clusters. In total, forty-three ESTs derived from different tissue sources and both EST databases were clustered into ten groups, one of these clusters represented hVRL-1. The remaining nine clusters have been named hVRa, hVRb, hVRc, hVRd, hVRe, hVRf, hVRg, hVRh and hVRi. For each EST the tissue source was assigned according to the annotations in the dbEST and Incyte databases. Since no obvious starting codon was present and the cluster sequences were shorter than the rat VR1 transcript none of these clusters were likely to represent a full-length vanilloid receptor transcript. Finally hVRg, hVRh and hVRi collapsed into a single contig. Sequence analysis has shown that

these cDNAs are likely to be chimeric. The 5' end has weak similarities with the rat VR1 gene but the 3' end is identical to a DNA binding protein. No more work was pursued with that transcript.

<u>**Reference Example B: Isolation of the human orthologue to the rat VR1 gene (reference examples B1-B4):**</u>

Reference Example B1: *In silico* assembly of human VR1

The consensus nucleotide sequences from the ten clusters were searched with the tBlastx program (20) against the rat VR1 sequences to identify the most likely open reading frames. Frame shifts were corrected when the sequence trace files were available. Each cluster was aligned against the rat VR1 amino-acid sequence according to the Blastx results. The Blastx alignment program (20) was used to compare the full-length rat VR1 protein with the amino-acid sequences of the ten clusters. The three clusters with the highest homology, displayed in figure 1, were aligned with the rat VR1 gene.

Cluster hVRa shared a high homology (70% identity and 75% similarity over a stretch of 107 amino acids) with the 5' of the rat VR1 sequence but did not seem to have a potential start codon. It contained two ESTs (EST1 and EST2) derived from the same tissue, bladder, and from the same patient. These two ESTs were selected for further investigation since this cluster was the most 5', had high homology with rat VR1 and the bladder tissue could be contaminated with sensory neurones. Both cDNA clones were ordered but only clone EST1 was received as EST2 failed the recovery procedure.

Cluster hVRb composed of two EST's (EST3 and EST4), with 89% identity and 92% similarity over 90 residues, showed the highest degree of homology to the rodent sequence. The overlap between both sequences was located towards the middle of the gene.

hVRc (EST5) also while having high homology (71% identity and 75% similarity over 65 residues) with rat VR1 was closely related to the C-terminus of the rat protein sequence.

Reference Example B2: Sequencing of clones

All DNA sequences were determined by automated DNA sequencing based on the dideoxy chain-termination method using the ABI 373A / 377 sequencers (Applied Biosystems). Sequence-specific primers were used with the 'Big-Dye' Terminator Cycle Sequencing kit (Applied Biosystems). The nucleotide sequence was analysed using programs from the University of Wisconsin Genetics Computer Group package.

More specifically when sequencing an EST clone, the following protocol was adopted. The EST1 clone was grown using standard procedures and DNA was isolated using Qiagen columns. SP6 (5' ATTTAGGTGACACTATAG) and T7 (5' TAATACGACTCACTATAGGG) primers flanking the cloning site were used to sequence both ends. Plasmid DNA (0.6 pmol) was used with 10.0 pmol of each primer for the dye terminator reaction. The SP6 end corresponded to the *in silico* derived EST sequence (identical to EST1). The T7 end did not have homologies with VR1 nor did it possess a long open reading frame or a polyadenylation motif. The size of the insert was determined by enzyme digestion of the DNA with the endonucleases NotI and EcoRI and calculated to be approximately 3kb.

Plasmid DNA (50ng) was used to amplify the insert by Polymerase Chain Reaction (PCR) with T7 and SP6 as primers. The PCR conditions included an initial hot-start at 94°C for 2 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 1 minute and terminated by 5 minutes at 72°C. The resulting PCR amplicon was separated on a 1.2% agarose gel and shown to be of ~3kb in size.

To fully sequence the PCR product the nuclease-Bal-31 technique was used where both strands of duplex DNA are degraded from both ends (23). After ethanol precipitation of the PCR product, the pellet was re-suspended in 30ml of 1X Bal-31 buffer (add buffer composition). A time-course digest with 2 units of Bal-31 enzyme (Roche Molecular Biochemicals) was carried out with 12 time points taken over 90 minutes (30 seconds, 1, 2, 3, 5, 7, 10, 15, 25, 45, 75 and 90 minutes). Three pools were made respectively from digests 1 to 4, 5 to 8 and 9

to 12. Each pool was blunt-ended and sub-cloned into the pCR-Script SK (+) plasmid from Stratagene at the SrfI site. After transformation, 16 colonies from each pool were screened by PCR with the flanking Reverse (5' GGAAACAGCTATGACCATG) and M13-20 (5' GTAAAACGACGGCCAGT) primers. The amplicons of 6 positive colonies per pool were subjected to direct sequencing (24) using the T3 (5' AATTAACCCTCACTAAAGGG) and T7 primers. The DNA sequences obtained were assembled using the GCG package, translated and aligned against the rat VR1 gene using the Blast tools. After analysis, the 3079bp amplicon was shown to have 2 introns of 603bp and 1221bp. The latter intron was located at the 3'end of the PCR product. The coding sequence covered 1255 bp and was separated by the former intron. Therefore the clone EST1 was likely to be a partially spliced and incomplete cDNA.

The clone belonging to cluster 1b (EST3) and derived from a kidney cDNA library was ordered and sequenced using the Bal-31 technique. After assembly of the sequences using the GCG package an identical overlap was identified with the DNA sequence of the cluster hVRc. Moreover a 3'end with a polyadenlyation signal and tail was identified. The complete sequence of the combined hVRb Bal-31 derived sequence and hVRc was 2063 bp (1020 bp of coding and 1043 bp of 3' untranslated sequence).

Reference Example B3: Amplification of the middle section of hVR1 using the Polymerase Chain Reaction

We formulated the hypothesis that both sequences (hVRa and hVRb/c) were part of a common transcript. If the human and rat VR1 were going to be similar, the 2 contigs should be separated by a gap of approximately 275bp. Primers were designed on both sides of the gap to amplify mRNA from brain tissues in order to clone the gap. A smear was obtained with the sense primer (5' TCTACTTCGGTGAACTGCCC) and antisense (5' ACGGCAGGGAGTCATTCTTC). For specificity 50ng of the PCR product were amplified with the nested sense (5' CTGCAGAACTCCTGGCAGA) and antisense (5' GTCACCACCGCTGTGGAAAA) primers. The 900bp nested amplicon was sequenced and shown to be identical to hVRa at one end and

hVRb/c at the oth r end. The middle part of the PCR product was homologous to the rat VR1 sequence. This region corresponded to 91 amino acids. When the sequences of hVRa, hVRb/hVRc and the internal amplicon are combined the total length of the Open Reading Frame (ORF) is 824 amino acids followed by a 3' untranslated sequence of 1043 bp. The human amino acid sequence is 87% identical to the rat sequence over that part of the coding region. This sequence was termed hVR1 because of its high degree of identity with the rat VR1 sequence.

Reference Example B4: Isolation of the 5' Terminus of hVR1 by PAC isolation

Since no start codon was identified at the 5' end an additional strategy was designed to identify the full-length sequence. Two primers, sense (5' TCCTCTGGCTTCCAACCCGTT) and antisense (5' GAACTGGGCAGAAAGTGCCT) were designed to amplify a 150bp product from the first intron mentioned in reference example B2. A P1 Artificial Chromosome (PAC) genomic clone (25) was isolated by PCR screening of a PAC library (Genome Systems, St Louis, Missouri). PAC DNA was recovered by using standard plasmid isolation protocol (26). An anti-sense primer was designed (5' CTGGAGTTAGGGTCTCCATCC) to sequence the genomic clone towards the potential 5' end of the gene. An open reading frame with a starting codon was identified. The gene structure was confirmed by using the GenScan software (27). The complete gene has a nucleotide sequence of 2517bp (figure 2) and encoded a 839 amino acid protein (Figures 3 and 4). The gene was named hVR1. Multiple alignment of the amino acid sequence of hVR1 and rat VR1 shows a remarkable degree of identity and similarities between both sequences (figure 5). The rVR1 and hVR1 amino acid sequences are 86% identical. Moreover after protein analysis 6 trans-membrane domains and 3 ankyrin binding domains were identified in hVR1 as in the rat VR1 gene.

<u>Example 1: Full-length Amplification of hVR1 from human DRG and assembly into cloning vectors</u>

HVR1 was PCR amplified in three sections from human DRG template. The 5' fragment was amplified using a sense primer encoding a NotI site and a strong

Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCGCCACCATGAAGAAATGGAGCAGCAC) and an antisense primer (5' AGGCCCACTCGGTGAACTTC). The thermo-cycling conditions used for this amplification included a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min. A final extension step of 72°C for 5 min completed the reaction. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle section of hVR1 was PCR amplified using the sense primer: 5' GACGAGCATGTACAATGAGA and antisense primer: 5' GTCACCACCGCTGTGGAAAA. The cycling conditions included a hot start at 94°C for 4 mins, followed by 35 cycles of 1 min at 94°C, 56°C and 72°C. A final extension step of 72°C for 5 min completed the reaction. A band of approximately 870 bp was excised from a 2 % agarose gel and cloned as detailed by the TOPO™ TA Cloning® kit into the vector pCR2.1®-TOPO. Finally the 3' end was PCR amplified with the sense primer: 5' TGTGGACAGCTACAGTGAGA and the antisense primer: 5'TGCACTGAATTCGAGCACTGGTGTTCCCTCAG which encoded an EcoRI site for cloning. The PCR conditions included a 90 sec hot start at 94°C followed by 35 cycles of 94°C for 50 sec, 50°C for 50 sec and 72°C for 50 sec. The cycling was completed with a 72°C step for 5 min. PCR products were separated on a 2% agarose gel and cloned into the vector pCR2.1®-TOPO.

Resulting clones for each of the three hVR1-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full length assembly of the gene. The NotI/DraIII (New England Biolabs) digested 5' end fragment ligated together with the middle DraIII/EcoRI fragment into a NotI/EcoRI restricted pBluescript SK (+) vector (Stratagene). Finally, the remaining 3' fragment was introduced into the resulting construct via MscI and EcoRI restriction sites, a map of the resulting construct is displayed in figure 6A.

Several clones were selected for sequence analysis to confirm that constructs still encoded the hVR1 consensus sequence. These were then digested with NotI/EcoRI and ligated into the mammalian expression vector pCIN5-new (a modified version of pCIN1 (32) having an IVS deletion as well as a 36 bp

deletion repositioning the start codon of neomycin phosphotransferase immediately after the upstream EMVC IRES) as illustrated in figure 6B.

Example 2: Chromosomal Localisation

The primers used to isolate the PAC clone (reference example B4) were selected for PCR on the G3 radiation hybrid panel from Stanford commercially available from Research Genetics (Huntsville, Alabama). The positive lanes and negative patterns were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR1 gene appears to be located on human chromosome 17 around marker SHGC-36073 (lod score=9.55).

Example 3: mRNA Distribution

The tissue distribution of hVR1 was established by slot-blot hybridisation. RNA was transferred onto a sheet of GeneScreen hybridisation transfer membrane (DUPONT) sandwiched in a slot blotter by suction via a vacuum pump. Once the membrane was rinsed in 2x SSC (3M sodium chloride and 0.3M sodium citrate pH7) for 2 min it was exposed to UV using an Ultraviolet crosslinker (Amersham Life Science) for 1min at 15000uW/cm^2 thus enabling cross-linkage of the RNA onto the membrane. The amounts of RNA on the blot are unknown. The probe was obtained by PCR amplification of a 260 bp product of the coding region of hVR1 with the following two primers: 5' TGTGGACAGCTACAGTGAGA and 5' GTGGAAAACCCGAACAAGA. Membranes were hybridised for 4 hr shaking at 60°C in a 10% dextran sulphate, 1% SDS (sodium dodecyl sulphate) and 1M NaCl solution. The probe was labelled with [α32P]dCTP (Amersham) using the Rediprime™DNA labelling system (Amersham), so as to obtain approximately 500,000cpm of the labelled probe per ml of prehybridisation solution. Briefly 100ng of probe was boiled for 3 minutes (denaturization) and then cooled on ice for 2 minutes in a total volume of 45µl. This was added to the labelling tube from the kit together with 3µl of 32P dCTP followed by an incubation at 37°C for 30 minutes. 400µl of Herring Sperm DNA (Sigma) at a concentration of 8µg/ml was added to the labelled probe and heated at 99°C for 3 minutes followed by rapid cooling on ice. The labelled probe was added and mixed well in pre-hybridisation solution. The membranes were hybridised overnight at 55°C.

The membranes were then washed, first at room temperature in 2xSSC and 1% SDS for 5 minutes, followed by 2x SSC and 1% SDS for 30 min at 50°C. If necessary further washes with 1x SSC and 0.5% SDS or 0.1xSSC and 0.1% for 30 mins at the same temperature were carried out. The membranes were then exposed to Scientific Imaging Film AR (Kodak) using intensifying screens at –70°C overnight and the film developed.

The results are shown on figure 7. Strong signals were observed with the positive controls (slots 4B and 5B). Signals are detected on the human DRG slots (1A and 1B). No signals were detected with the water control (slot 3B). Three multi-tissue northern blots (Clontech) with a wide range of tissues have also been hybridised with the same probe, however no signals were detected. RT-PCR was performed on various tissues with the primer combination used to amplify the probe. A strong band was detected in DRG RNA. Taken together these hybridisations suggest that hVR1 is specifically expressed in neuronal tissue and DRG in particular.

Example 4: Design and production of Anti-hVR1 Antibody

The peptides CHIFTTRSRTRLFGKGDSEEASC (peptide68) and CGSLKPEDAEVFKDSMVPGEK (peptide69) were synthesised by standard solid phase techniques and purified by gel filtration chromatography. These peptides were conjugated via their Cys residues to the carrier protein, Tuberculin PPD (purified protein derivative) using sulpho-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexan-1-carboxylate). Rabbits, previously sensitised to Bacillus Calmette Guerin (BCG), were inoculated with the resulting conjugates emulsified in incomplete Freund's adjuvant at approx monthly intervals. Serum was prepared from blood samples taken 7 days after each immunisation. The specific antibody response was followed by indirect enzyme-linked immunosorbent assay (ELISA) using free peptide as antigen. Immunoglobulins were purified from high titre sera using immobilsed peptide affinity columns (sulpholink Pierce). Rabbits designated M143, 144 and 145 received peptide68 conjugate, rabbits M146, 147 and 148, peptide69 conjugate.

The antibodies have been validated by specific staining of the recombinant protein expressed in HEK293 cells. Whole cell lysates were prepared in Sample

Buffer (4 ml dH_2O, 1 ml 0.5 M Tris-HCl, pH 6.8, 0.8 ml glycerol, 1.6 ml 10 % w/v SDS, 0.4 ml 2-β mercaptoethanol and 0.2 ml of 0.05 % w/v bromophenol blue) and proteins separated by SDS-PAGE and transferred to a nitrocellulose filter by electroblotting. Following incubation with the antisera, bound immunoglobulins were revealed using HRP-conjugated secondary antibodies and enhanced chemiluminescence (ECL) detection. The antisera showed specific binding to a protein(s) of the appropriate molecular weight(s) in extracts of VR1 transfected cells, but not in control extracts, this is illustrated in figure 8.

Example 5: *Insitu* localisation of hVR1 using specific antibody

The purified immunoglobulins have been used for immunohistochemical staining of rat DRG tissue sections. Fixed cryosections of DRG were incubated with antibodies for 48h at 4°C at concentrations between 0.1 to 0.5μg/ml. Following a washing step, bound antibodies were detected by indirect immunofluorescence. The antibodies recognised exclusively small diameter cell bodies of the peripheral sensory neurones as displayed in figure 9. This observation has been extended to human DRG tissues for the anti-peptide68 peptide antibodies demonstrating cross-reactivity with the human sequence as expected. Figure 10A demonstrates labelling of DRG cell bodies with an arrow that points to small diameter neuronal cell body) and in figure 10B the arrow points to labelled neurones innervating human skin.

Example 6: Mammalian Cell Expression (examples 6a-6b)

Example 6a: Transient expression of hVR1 in mammalian cells

HEK293 cells were plated onto a 6 well plate, containing poly-l-lysine coated coverslips, at 5 x 10^4 cells per well. Next day, fresh media was added to the cells (50% confluent). CalPhos Mammalian Transfection Protocol (Clontech, K2051-1) was used for DNA transfection. For each well of cells, solution A was made up containing 8ug hVR1pCIN5, 2μg pEYFP-N1 reporter DNA, 12.4 μl calcium solution and water to 100μl. Solution B (hepes buffered saline) was slowly vortexed while solution A was added dropwise. The mixture was incubated at room temperature for 20 minutes, and then added to cells. The plate was slowly rocked to distribute the solution. The cells were incubated at 37°C for 5 hours, and then washed with phosphate buffered saline. Fresh culture

medium was added and the plate was incubated 24-48 hours for functional analysis.

Example 6b: Stable expression of hVR1 in mammalian cells

HEK293 cells were plated onto a 6 well plate at 1 x 10^5 cells per well. Next day, fresh media was added to the cells (50% confluent). CalPhos Mammalian Transfection Protocol (Clontech, K2051-1) was used for DNA transfection. For each well of cells, solution A was made up containing 2μg hVR1pCIN5, 12.4μl 2M calcium solution and water to 100μl. Solution B (hepes buffered saline) was slowly vortexed while solution A was added dropwise. The mixture was incubated at room temperature for 20 minutes, and then added to cells. The plate was slowly rocked to distribute the solution. The cells were incubated at 37°C for 5 hours, and then washed with phosphate buffered saline. Fresh culture medium was added and the plate was incubated 48 hours at 37°C, 5% CO_2. Cells were harvested into 100mm dishes in selection medium containing 800μg/ml geneticin. Cells were then incubated and fed at 4 day intervals. In total around 10 days selection is required for each single cell to multiply into a visible clone. Well-separated clones were each picked (with a gilson tip) into separate wells of a 96 well plate, containing maintenance medium (400μg/ml geneticin). Cells were expanded into flasks for freezing stocks and functional analysis. Stable cells may be plated at 1 x 10^5 cells onto poly-l-lysine coated coverslips in 6 well plate, for calcium imaging next day.

Example 7: Functional Analysis of hVR1(examples 7a-7c):

Example 7a: Electrophysiology using patch clamp methods

The activation of human VR-1 channels transiently expressed in HEK293T cells by capsaicin was investigated. Cells grown on poly-L-lysine-coated glass coverslips were placed in a recording chamber (0.5ml) and superfused with extracellular solution (2ml min^{-1}). The extracellular solution contained: NaCl (140mM), KCl (5mM), MgCl2 (2mM), CaCl2 (2mM), 4-(2-hydroxethyl)-1-piperazineethanesulphonic acid (HEPES, 10mM) and glucose (10mM). The pH was adjusted to 7.4 with NaOH and osmolarity ranged from 310-320mOsm l^{-1}. Patch pipettes (borosilicate glass) were pulled using a Sutter P-97 electrode puller. The pipettes were filled with an internal solution consisting of: CsCl

(140mM), ethylene glycol-bis(β-aminoethyl ether) *N,N,N',N'*-tetra acetic acid Cs salt (Cs-EGTA, 5mM) and HEPES (10mM). The pH was adjusted to 7.25 using CsOH and the osmolarity ranged from 275-290 mOsm. When filled with this internal solution, patch electrodes had resistances of 2-5 MΩ. Currents were recorded using standard whole-cell voltage clamp recording techniques (31) at room temperature (21-23°C) using an Axopatch 200A amplifier and signals were sampled at 2 or 0.1 kHz. The majority of series resistance errors (80-85%) were minimized with compensation circuitry. Membrane potentials were not corrected for junction potentials (<4 mV). Voltage pulses and data collection were performed on-line using pClamp8 software (Axon Instruments) interfaced with amplifiers. Membrane potentials were maintained at –60mV between protocols.

Capsaicin or capsazepine (CPZ) were applied, using a 'fast-flow sytem', directly onto the recording cell (<1s to equilibrate). The effects of capsaicin were measured either by application during constant recording while holding the membrane potential at –60mV to elicit an inward current, or applying voltage ramps (-100 to +60mV) in the absence and presence of capsaicin. Similarly both these methods of recording currents evoked by the application of capsaicin were used to demonstrate the blockade by the antagonist (CPZ).

Figure 11A reveals that application of capsaicin (1 μM), on human VR1 channels transiently expressed in HEK293T cells, produces an inward current when the membrane was held at a potential of -60mV. This response was abolished by 1μM CPZ and the blockade was partially reversible.

In the presence of 1 μM capsaicin, voltage ramps (-100 to +70mV) produced a current-voltage relationship demonstrating a substantial outward rectification. Addition of 1μM CPZ completely blocked the current (figure 11B). Again, only partial recovery was observed, especially for the inward currents evoked by negative potentials.

Capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium is illustrated in figure 12. Voltage ramps (-100 to +70) were applied and the addition of capsaicin (1μM) evoked an outwardly rectifying current. Repeated additions of capsaicin resulted in a progressive 'rundown' in

the size of the response (figure 12A). Figure 12B shows a plot of the current elicited at a potential of +65mV against time illustrating the 'rundown' in current amplitude. Voltage ramps were applied every 20s and capsaicin added at 2min intervals for approximately 40s. By the 6th addition the current had reduced about 4-fold.

When the external calcium was replaced with 5mM EGTA the size of the current increased dramatically (figure 12C). However, when calcium was re-applied to the external solution, the current evoked by capsaicin (1μM) was approximately equivalent to that of the 6th addition shown in (figure 12A).

Example 7b: Calcium Imaging with HEK293 expressing hVR1

HEK293 cells expressing hVR1 transiently or stably, were plated onto poly-l-lysine coated cover slips at 1 x 10^5 cells per well. They were analysed on the following day by calcium imaging (QuantiCell 700, Applied Imaging). On the day of experiment, WASH buffer was prepared by adding $CaCl_2$ to extracellular medium (ECM) to a final concentration of 2mM, (ECM contains 125mM NaCl, 5mM KCl, 2mM $MgCl_2$, 0.5mM NaH_2PO_4, 5mM $NaHCO_3$, 10mM Hepes, 10mM glucose, 0.1% BSA, pH7.4). The calcium sensitive dye solution was prepared by adding 50μl 5% pluronic F-127 in DMSO (Molecular Probes) to a vial of fura2-AM (Molecular Probes). After mixing, 20μl of the fura2-AM solution was added to 10ml WASH. 1.5 ml was then added to cells, which were then incubated at 37°C for 30 minutes. The plate was washed three times with WASH. 1ml WASH was added and stored in dark. Agonists and antagonists were prepared in WASH at 5x their required assay concentrations. The reagents and assay temperature was kept at 37°C. For the transiently transfected cells, the YFP reporter DNA fluorescence (490nm excitation) was used to identify the transfected cells. Cells were initially imaged in 400μl WASH (or 300μl WASH plus 100μl antagonist e.g. capsazepine). After approximately 1 min, 100μl agonist (e.g. capsaicin, anadamide or resiniferatoxin) at 5 x the desired concentration was added to give final 1x concentration. A sequence of images (340/380nm excitation) were taken to monitor calcium influx response in cells before (30-60 secs), and after the addition of agonist (2-5 mins). Figure 13 displays time courses taken for each of the tests set up to look at the affect of the different agonists mentioned above in the presence or absence of the rat VR1 antagonist, capsazepine. The Imager

[redacted]

also plots graphs of respective calcium concentration (nM) versus time (seconds) as shown in figure 14. After the addition of agonist (e.g. capsaicin, indicated by the vertical arrow on graph), the cells expressing hVR1 are stimulated to influx calcium. This is shown by the appearance of peak on the trace. The peak height correlates with hVR1 expression level. Varying levels of expression is some times seen depending on which cells are selected for the graph. Similar experiments may be accomplished to examine the response of protons and heat.

Example 7c: Use of a FLIPR assay with VR1

FLIPR (Fluorometric Imaging Plate Reader) is a high throughput fluorescence-based drug discovery tool for functional cell analysis. Intracellular calcium is monitored with the calcium sensitive dye, fluo3-AM. HEK293 cells stably expressing rat VR1 were plated into a 96 well, poly-l-lysine treated FLIPR plate at 3 x 10^4 cells per well. On the following day, the plate was processed for FLIPR. FBP buffer was prepared (15μM Probenecid (calcium ATPase pump blocker) in 1x FLIPR buffer (145mM NaCl, 5mM KCl, 1mM MgCl2, 2mM $CaCl_2$, 10mM glucose, 20mM Hepes). FBP buffer pH was then adjusted to 7.4 with NaOH. 400μl DMSO was added to a vial of fluo3-AM (Cambridge Bioscience, F-1241). The fluo3-AM solution was incubated at 37°C for 10 min and vortexed. LOAD was prepared by adding 20μl of fluo3-AM solution and 20μl 20% pleuronic F-127 in DMSO (Cambridge Bioscience, P-3000) into 10 ml FBP. The 96 well plate containing cells was flicked off to remove cell medium. 100μl LOAD was added per well. Cells were then incubated at 37°C for 60 minutes. Capsaicin (a rVR1 agonist) and capsazepine (CPZ, a rVR1 antagonist) were prepared at 10x the desired final assay concentrations in FBP. The plate was flicked to remove LOAD from cells, and 180μl FBP was added per well. The FLIPR machine added 20μl capsaicin per well to give a final 1x concentration. Cells were monitored for 70 seconds after agonist addition. The FLIPR traces (fluorescence change (counts) versus time (seconds)) were produced for each well. Peaks indicate capsaicin-gated calcium influx, by cells expressing rVR1. The peak height correlates with the rVR1 expression level. To measure antagonism of the VR1 response 20μl 10x antagonist CPZ was added into wells to give a final 1x concentration. The plate was incubated for 15 minutes at room temperature prior reading in the FLIPR. The FLIPR traces recorded for each well show that the

peak heights are reduced in cells pre-incubated in CPZ. The same FLIPR assay may be used to monitor the response of human VR1 on exposure to agonists and antagonists.

Example 8: Example of a screen using human VR1.

FLIPR assay technology may be utilised to screen for hVR1 modulators according to the procedure described in figure 15. Human VR1 may be gated with protons, capsaicin or heat.

Reference Example C: Identification and partial characterisation of additional human vanilloid receptors (referenence examples C1-C3):

Reference Example C1: Identification and characterisation of a novel vanilloid–like receptor, hVR3

ESTs belonging to the remaining clusters were characterised by *in silico* cloning (reference example A). The following clones were used during this process: -EST6/EST7 (hVRd), -EST8. (hVRe), - EST9/EST10. (hVRf). These EST clusters have been aligned with rat VR1 in figure 16, note that this diagram is not to scale.

Reference Example C2: Sequencing of clones

Further sequencing, as detailed in reference example B2, and *in silico* cloning, enabled clusters hVRd, hVRe and hVRf to collapse forming a single contig of 583 amino acids. This sequence was named hVR3 and has 49 % identity with the rat VR1 sequence. It was unlikely that this single contig was a full-length vanilloid receptor transcript as no obvious starting codon was present and it was shorter than the rat VR1 transcript.

Reference Example C3: Identification of the 5' terminus of hVR3

Two primers (sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG) designed to PCR amplify an amplicon stretching the 3' end of hVR3 and its 3'utr were used to isolate a genomic PAC clone (Genome Systems. St Louis, Missouri). The hVR3 specific PAC clone was then used as template to generate a library. This was achieved by sonicating 6μg of Qiagen purified PAC construct, size selecting fragmented DNA of 500-

2000bp. These resulting fragments were then blunt ended and cloned into the vector pCR®-Blunt as detailed in the manufacturers protocol supplied with the Zero Blunt™ PCR cloning kit (Invitrogen). Clones were then sequenced (reference example B2) to identify the complete 5' end of the hVR3 transcript. The full-length nucleotide sequence of the hVR3 gene is displayed in figure 17. Figure 18 illustrates both nucleotide and encoded amino acid sequence of the human VR1 and figure 19 depicts the amino acid sequence of the hVR3 gene with shaded regions denoting predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Example 9: Full-length Amplification of hVR3 from human kidney template

Human kidney was used as a source of template for the PCR amplification of hVR3. Primers used for amplification were designed to isolate the gene in three fragments. Primers designed to isolate the 5' end included a sense primer encoding a NotI site and a strong Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCGCCACCATGCCCAGGGTAGTTGGAC and antisense primer (5' CACCTCTTGTTGTCACTGGA). The PCR conditions used were a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and finally one cycle at 72°C for 5 min. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle fragment was PCR generated using sense and antisense primers 5' CAAATCTGCGCATGAAGTTCCAG and 5' GCCACGAGAAGTTCCACGTAGTG respectively in the presence of 5% DMSO. PCR thermo-cycling required 35 cycles of 1 min at 94°C, 58°C and 72°C for successful amplification of the fragment which was then excised from a 2% agarose gel for cloning into the pCRII®-TOPO vector. Finally the 3' fragment was amplified with a sense primer 5' GCTGCTCCCATTCTTGCTGA and an antisense primer 5' TGCACTCTCGAGAAATGAGTGGGCAGAGAAGC encoding a XhoI restriction site. This fragment was successfully amplified using a hot start at 94°C for 4 min followed by 35 cycles of 94°C for 50 sec, 48°C for 50 sec and 72°C for 2 min. The cycling was completed with a 72°C step for 5 min. The amplified fragment was excised from a 2% agarose gel and clone into the pCRII®-TOPO vector.

Resulting clones for each of th three PCR generated hVR3-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full-length assembly of the gene. The DraIII restriction site of the pBluescript SK (+) vector (Stratagene) was firstly abolished by digestion with DraIII followed by a blunt ending step using T_4 DNA polymerase (New England Biolabs). This modified vector was then restricted to enable the ligation of both a NotI/NcoI 5' fragment and NcoI/ EcoRI middle fragment. Finally, the remaining 3' fragment was introduced into the resulting construct via DraIII and XhoI sites (figure 20A).

Several clones were selected for sequence analysis to confirm that the constructs still encoded the hVR3 consensus sequence. These were then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1 (+) (Invitrogen) as seen in figure 20B. The resulting hVR3 consensus sequence is shown in the multiple alignment along with the full-length sequence of hVR1 and the published hVRL-1 in figure 21.

Example 10: Chromosomal localisation

The 3' terminus, including the 3' UTR sequence of hVR3 was used to design two primers to amplify a product of 360 bp: sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG. The G3 radiation hybrid panel from Stanford University (Research Genetics, Huntsville, Alabama) was screened by PCR. The positive and negative lanes were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR3 gene appears to be located on human chromosome 12 around markers D12S177E (lod score=15) and D12S1893 (lod score=14).

Example 11: mRNA distribution

The following primers (5' ACAAGAAGGCGGACATGCGG and 5' ATCTCGTGGCGGTTCTCAAT) were used to obtain a PCR product from the coding region of hVR3. This amplicon was used as a probe on multi-tissue northern blots, the protocol of which is detailed in example 3, to determine the tissue distribution of the gene (figures 22A, 22B and 22C). A transcript of approximately 3.8 kb was detected in the following tissues (the intensities of the

signals are indicated in brackets): trachea (very strong), kidney (strong), pancreas (strong), prostate (strong), placenta (strong), bone marrow (weak), adrenal gland (weak), lymph node (weak), spinal cord (weak), thyroid (weak), stomach (weak), lung (weak) and liver (weak).

Since these commercial blots (Clontech, Palo Alto, California, USA) should have the same amount of RNA it is interesting to note the very strong signal in the trachea lane (figure 22A). This could indicate the potential of hVR3 as a target for respiratory pathologies. It was shown by RT-PCR with the primer combination used to produce the probe that the gene is not expressed in DRG.

Example 12: Riboprobe generation for the in situ localisation of hVR3

The same probe, which was specific to hVR3 in Northern blot analysis (example 11), was used to generate a riboprobe. This hVR3 specific probe was cloned into the T7 and SP6 encoding pCRII®-TOPO vector (Invitrogen). This construct was then used in the *in vitro* transcription of DIG labelled RNA strands from the vectors promoters as described in the manufacturers instructions as detailed in the DIG RNA labelling kit (Roche Molecular Biochemicals). This riboprobe may be used to identify the cellular localisation of hVR3 present in tissues such as trachea, lung, pancreas, prostate, placenta and kidney.

References

1. Szallasi, A. and Blumberg, P.M (1993) Mechanisms and therapeutic potential of vanilloids (capsaicin-like molecules). *Adv. Pharmacol.*, 24, 123-155.

2. Jansco, G. (1968) Desensitisation with capsaicin and related acylamides as a tool for studying the function of pain receptors. In: K. Lin, D. Armstrong and E.G. Pardo (Eds), Pharmacology of Pain, Pergamon Press, Oxford, 33-55.

3. Szolcsanyi, J. (1993) In *Capsaicin in the study of pain.* Ed. J. Wood, J. London: Academic Press, 1-26.

4. Szallasi, A. and Blumberg, P.M. (1996) Vanilloid receptors: new insights enhance potential as a therapeutic target. *Pain*, 68, 195-208.

5. Jansco, G., Kiraly, E. and Jansco-Gabor, A. (1977) Pharamacologically induced selective degeneration of chemosensitive primary sensory neurons. *Nature*, 270, 741-743.

6. Oh, U., Hwang, S.W. and Kim, D. (1996) Capsaicin activates a nonselective cation channel in cultured neonatal rat dorsal root ganglion neurons. *J. Neurosciences*, 16, 1659-1667.

7. Wood, J.N et al. (1988) Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *J. Neurosciences*, 8, 3208-3220.

8. Szolcsanyi, J. and Jansco-Gabor, A. (1975) Sensory effects of capsaicin congeners I. Relationship between chemical structure and pain-producing potency of pungent agents. *Drug Res.*, 25, 1877-1881.

9. Szolcsanyi, J. and Jansco-Gabor, A. (1976) Sensory effects of capsaicin congeners II. Importance of chemical structure and pungency in desensitising activity of capsaicin-like compounds. Drug res., 26, 33-37.

10. James, I.F., Nikina, N. and Wood, J.N. (1993) The capsaicin receptor. In *Capsaicin in the Study of Pain*. Ed. Wood, J. London: Academic Press, 83-104.

11. Szallasi, A. and Blumberg, P.M. (1990) Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. *Brain Research*, 524, 106-111.

12. Bevan, S., Hothi, S., Hughes, G., James, I.F., Rang, H.P., Shah, K., Walpole, C.S.J. and Yeats, J.C. (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *British Journal of Pharmacology*, 101, 423-431.

13. Szallasi, A., Blumberg, P.M., Nilsson, S., Hokfelt, T. and Lundberg, J.M. (1994) Vizualisation by [^{3}H] resiniferatoxin autoradiography of capsaicin-sensitive neurons in the rat, pig, and man. *European Journal of Pharmacology*, 264, 217-221.

14. Szallasi, A., Nillson, S., Farkas-Szallasi, T., Blumberg, J.M., Hokfelt, T. and Lundberg, J.M. (1995) Vanilloid (capsaicin) receptors in the rat: distribution in the brain, regional differences in the spinal cord, axonal transport to the periphery, and depletion by systemic vanilloid treatment. *Brain Research*, 703, 175-183.

15. Caterina, M.J., et al. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, 389, 816-824

16. Bevan, S. and Gepetti P. (1994) Protons: small stimulants of capsaicin-sensitive sensory nerves. *Trends in Neurociences*, 17, 509-512.

17. Petersen, M. and LaMotte, R.H. (1993) Effect of protons on the inward current evoked by capsaicin in isolated dorsal root ganglion cells. *Pain*, 54, 37-42.

18. Kress, M. Fetzer, S., Reeh, P.W. and Vyklicky, L. (1996) Low pH facilitates capsaicin responses in isolated sensory neurons of the rat. *Neurosciences Letters*, 211, 5-8.

19. Wilcox, A.S., Khan, A.S., Hopkins, J.A., and Sikela, J.M. (1991). Use of 3' untranslated sequences of human cDNAs for rapid chromosome assignment and conversion to STSs: Implications for an expression map of the genome. *Nucleic Acids Res.*, 19, 1837-1843.

20. Altschul, S.F., Warren, G., Webb, M., Myers, E.W., and Lipman, D.J., (1990). Basic local alignment search tool. *J. Mol. Biol.*, 215, 403-410.

21. Boguski, M.S., Lowe, T.M. and Tolstohev, C.M. (1993) dbEST: database for 'Expressed Sequence Tags' Nature Genetics, 4, 332-333.

22. Gill, R.W., Hodgman, C.T., Littler, C.B., Oxer, M.D., Montgomery, D.S., Taylor, S. and Sanseau, P. (1997). A new dynamic tool to perform assembly of Expressed Sequence Tags (ESTs). *Computer Applications in Biosciences (CABIOS)*, 13, 453-457.

23. Lau, P.P. and Gray H.B. (1979). *Nucleic Acids Research*, 6, 331.

24. Trower MK, Burt D, Purvis IJ, Dykes CW & Christodoulou C (1995)

28. Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: a Laboratory Manual. 2nd Edition. CSH Laboratory Press. (1989)

29. Davis L. G., Battey J. F. & Dibner M.D., Basic Methods in Molecular Biology. 1st Edition. Elsevier (1986).

30. Caterina, M. C., Rosen, T. A., Tominaga, M., Brake, A. J. & Julius, D. (1999) A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature*, 398,436-441.

31. Hamill, O.P. Marty, A., Neher, E., Sakmann, B., & Sigworth, F.J. (1981). Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, 391, 85-100.

32. Rees. S., Coote, J., Stables, J., Goodson, S., Harris, S. & Lee, M. G. (1996) Bicistronic vector for the creation of stable mammalian cell lines that predisposes all antibiotic-resistant cells to express recombinant protein. Biotechniques, 20, 102-110.

Claims

1. An isolated human vanilloid receptor (hVR) protein or a variant thereof.

2. An isolated human vanilloid receptor (hVR) protein according to claim 1 which is hVR1 or a variant thereof.

3. An isolated human vanilloid receptor (hVR) protein according to claim 1 which is hVR3 or a variant thereof.

4. An isolated human vanilloid receptor (hVR) protein according to claim 2 having an amino acid sequence as shown in Figure 3.

5. An isolated human vanilloid receptor (hVR) protein according to claim 3 having an amino acid sequence as shown in Figure 18.

6. A nucleotide sequence encoding a human vanilloid receptor (hVR) protein or a variant thereof, or a nucleotide sequence which is complementary thereto.

7. A nucleotide sequence according to claim 6 encoding for an hVR1 protein or a variant thereof, or a nucleotide sequence which is complementary thereto.

8. A nucleotide sequence according to claim 6 encoding for an hVR3 protein or a variant thereof, or a nucleotide sequence which is complementary thereto.

9. A nucleotide sequence according to claim 6 which is a cDNA sequence.

10. A nucleotide sequence according to claim 7 which is a cDNA sequence

11. A nucleotide sequence according to claim 8 which is a cDNA sequence

12. A nucleotide sequence according to claim 7 as shown in Figure 2.

13. A nucleotide sequence according to claim 8 as shown in Figure 17.

14. An expression vector comprising a nucleotide sequence according to any one of claims 6 to 13, which is capable of expressing an hVR protein or a variant thereof.

15. An expression vector according to claim 14 which is capable of expressing an hVR1 protein or a variant thereof.

16. An expression vector according to claim 14 which is capable of expressing an hVR3 protein or a variant thereof.

17. A stable cell line comprising an expression vector according to claim 14.

18. A stable cell line comprising an expression vector according to claim 15.

24. An antibody according to claim 23 which is specific for hVR1 or a variant thereof.

25. An antibody according to claim 23 which is specific for hVR3 or a variant thereof.

26. A method for identification of a compound which exhibits hVR modulating activity comprising contacting a human vanilloid receptor (hVR) protein or a variant thereof according to any one of claims 1 to 5 with a test compound and detecting modulating activity or inactivity.

27. A compound which modulates hVR activity, identifiable by a method according to claim 26.

28. A compound according to claim 27 for use in therapy.

29. The use of a compound according to claim 27 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

30. The use according to claim 28 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

31. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 27.

32. A method according to claim 31 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain,

rheumatoid arthritic pain, neuropathies, neuralgia, alg sia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

33. A compound which modulates hVR activity, identifiable by a method according to claim 26, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β-acaridial, scutigeral, merulidial, anandamide and capsazepine.

34. A compound according to claim 33 for use in therapy.

35. The use of a compound according to claim 33 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

36. The use according to claim 35 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

37. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 33.

38. A method according to claim 37 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a

urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

39. A compound identified by the method according to claim 26.

40. A compound according to claim 39 for use in therapy.

41. The use of a compound according to claim 39 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

42. The use according to claim 41 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

43. A method of treatment or prophylaxis of a disorder which is responsive

a variant thereof, under conditions suitable for obtaining expression of the hVR protein or variant thereof.

46. A method of producing an hVR1 protein or a variant thereof comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR1 protein or a variant thereof, under conditions suitable for obtaining expression of the hVR1 protein or variant thereof.

47. A method of producing an hVR3 protein or a variant thereof comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR3 protein or a variant thereof, under conditions suitable for obtaining expression of the hVR3 protein or variant thereof.

48. A human vanilloid receptor (hVR) protein or a variant thereof for use in a method of screening for agents useful in the treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient

49. A human vanilloid receptor (hVR) protein according to claim 48 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

50. A human vanilloid receptor (hVR) protein according to claim 48 or 49 which is hVR1 or a variant thereof.

51. A human vanilloid receptor (hVR) protein according to claim 48 or 49 which is hVR3 or a variant thereof.

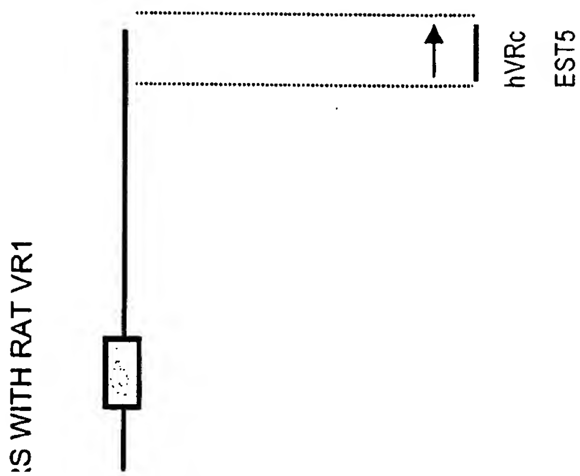

hVRc
EST5
S WITH RAT VR1

FIG. 2

hVR1 SEQUENCE INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt 1 TO 2517) AND 3'UTR (nt 2518 TO nt 3560)

```
-773  cccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca  -714

-713  aaggccagaagcttgacagatgttgattcataaaatgcaaaagccaaaatccaaaatct  -654

-653  tgtataagctcagtggctgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg  -594

-593  ctgatgatgtgtggacccgttgcacagcagggcccgcagtgcggtgtgggtgtgggtggg  -534

-533  ccagtctctgccgctcaccctattccagggacacagtctgcttggctcttctggactgag  -474

-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccaccccggccgtttt  -414

-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354

-353  gcacctgaggcccagagacgaggagggatgggtctaacccagaaccacagatggctctga  -294

-293  gccgggggcctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234

-233  gctaggcctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174

-173  gttctaggggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114

-113  ccttggcaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54

 -53  ccggcgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAG  6

   7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66

  67  GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126

 127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186

 187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC  246

 247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
```

```
307   GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT   366

367   GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG   426

427   CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC   486

487   ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG   546

547   CAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGC   606

607   CAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTGGTG   666

667   GAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGG   726

727   CGGCCTGGATTCTACTTCGGTGAACTGCCCCTGTCCCTGGCCGCGTGCACCAACCAGCTG   786

787   GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGAC   846

847   TCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAAC   906

907   ACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAACTGCACCCG   966

967   ACGCTGAAGCTGGAGGAGCTCACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCT   1026

1027  GGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG   1086

1087  TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCGTGCACTCCTCGCTG   1146

1147  TACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTAC   1206

1207  AGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTC   1266

1267  CTGCAGGACAAGTGGGACAGATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTAC   1326

1327  TGCCTGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCT   1386

1387  CCCTTTAAGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTG   1446
```

FIG. 2CONT'D

1447 TTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTCAGTATTTCCTGCAGAGGCGGCCGTCG 1506

1507 ATGAAGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTC 1566

1567 ATGCTGGCCACCGTGGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTA 1626

1627 TTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATG 1686

1687 GGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTT 1746

1747 GTCTACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGG 1806

1807 AAGAATGACTCCCTGCCGTCTGAGTCCACGTCGCACAGGTGGCGGGGGCCTGCCTGCAGG 1866

1867 CCCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACC 1926

1927 ATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCATC 1986

1987 CTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTC 2046

2047 ATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGA 2106

2107 GCCATCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGC 2166

2167 TCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGC 2226

2227 TTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAA 2286

2287 GACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCTGAGCTTCTCCCTGCGGTCAAGCAGA 2346

2347 GTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCT 2406

2407 CGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTG 2466

2467 AAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGtgaggacgt 2526

2527 cacgcagacagcactgtcaacactgggccttaggagaccccgttgccacggggggctgct 2586

FIG.2CONT'D

2587 gagggaacaccagtgctctgtcagcagcctggcctggtctgtgcctgcccagcatgttcc 2646

2647 caaatctgtgctggacaagctgtgggaagcgttcttggaagcatggggagtgatgtacat 2706

2707 ccaaccgtcactgtccccaagtgaatctcctaacagactttcaggtttttactcacttta 2766

2767 ctaaacagtttggatggtcagtctctactgggacatgttaggcccttgttttctttgatt 2826

2827 ttattcttttctgtgagacagagttcactcttgttgcccaggctggagtgcagtggtgtg 2886

2887 atcttggctcactgcaacctctgctcccgggttcaagcgattcttctgcttcagtctccc 2946

2947 aagtagcttggattacaggtgagcactaccacgcccggctaattttgtatttttaatag 3006

3007 agacggggtttcaccatgttggccaggctggtctcgaactcttgacctcaggtgatctgc 3066

3067 ccgccttggcctcccaaagtgctgggattacaggtgtgagccgctgcgctcggccttctt 3126

3127 tgattttatattattaggagcaaaagtaaatgaagcccaggaaaacacctttgggaacaa 3186

3187 actcttcctttgatggaaaatgcagaggcccttcctctctgtgccgtgcttgctcctctt 3246

3247 acctgcccgggtggtttgggggtgttggtgtttcctccctggagaagatgggggaggctg 3306

3307 tcccactcccagctctggcagaatcaagctgttgcagcagtgccttcttcatccttcctt 3366

3367 acgatcaatcacagtctccagaagatcagctcaattgctgtgcaggttaaaactacagaa 3426

3427 ccacatcccaaaggtacctggtaagaatgtttgaaagatcttccatttctaggaacccca 3486

3487 gtcctgcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataa 3546

3547 acagatatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 3591

FIG. 2CONT'D

FIG. 3

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR1 INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt TO 2517) AND 3'UTR (nt 2518 TO nt 3560)

```
-773  cccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca  -714

-713  aaggccagaagcttgacagatgttgattcataaaatgcaaaagccaaaatccaaaatct  -654

-653  tgtataagctcagtggctgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg  -594

-593  ctgatgatgtgtggacccgttgcacagcagggcccgcagtgcggtgtgggtgtgggtggg  -534

-533  ccagtctctgccgctcaccctattccagggacacagtctgcttggctcttctggactgag  -474

-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccaccccggccgtttt  -414

-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354

-353  gcacctgaggcccagagacgaggagggatgggtctaacccagaaccacagatggctctga  -294

-293  gccgggggcctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234

-233  gctaggcctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174

-173  gttctagggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114

-113  ccttggcaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54

 -53  ccggcgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAG  6
   1                                                        M  K    2

   7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66
   3  K  W  S  S  T  D  L  G  A  A  A  D  P  L  Q  K  D  T  C  P    22

  67  GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126
  23  D  P  L  D  G  D  P  N  S  R  P  P  P  A  K  P  Q  L  S  T    42

 127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186
  43  A  K  S  R  T  R  L  F  G  K  G  D  S  E  E  A  F  P  V  D    62

 187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC  246
  63  C  P  H  E  E  G  E  L  D  S  C  P  T  I  T  V  S  P  V  I    82

 247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
  83  T  I  Q  R  P  G  D  G  P  T  G  A  R  L  L  S  Q  D  S  V    102

 307  GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT  366
 103  A  A  S  T  E  K  T  L  R  L  Y  D  R  R  S  I  F  E  A  V    122

 367  GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG  426
 123  A  Q  N  N  C  Q  D  L  E  S  L  L  L  F  L  Q  K  S  K  K    142

 427  CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC  486
 143  H  L  T  D  N  E  F  K  D  P  E  T  G  K  T  C  L  L  K  A    162

 487  ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG  546
```

163 M L N L H D G Q N T T I P L L L E I A R 182

547 CAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGC 606
183 Q T D S L K E L V N A S Y T D S Y Y K G 202

607 CAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTGGTG 666
203 Q T A L H I A I E R R N M A L V T L L V 222

667 GAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGG 726
223 E N G A D V Q A A A H G D F F K K T K G 242

727 CGGCCTGGATTCTACTTCGGTGAACTGCCCCTGTCCCTGGCCGCGTGCACCAACCAGCTG 786
243 R P G F Y F G E L P L S L A A C T N Q L 262

787 GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGAC 846
263 G I V K F L L Q N S W Q T A D I S A R D 282

847 TCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAAC 906
283 S V G N T V L H A L V E V A D N T A D N 302

907 ACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAACTGCACCCG 966
303 T K F V T S M Y N E I L I L G A K L H P 322

967 ACGCTGAAGCTGGAGGAGCTCACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCT 1026
323 T L K L E E L T N K K G M T P L A L A A 342

1027 GGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG 1086
343 G T G K I G V L A Y I L Q R E I Q E P E 362

1087 TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCGTGCACTCCTCGCTG 1146
363 C R H L S R K F T E W A Y G P V H S S L 382

1147 TACGACCTGTCCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTAC 1206
383 Y D L S C I D T C E K N S V L E V I A Y 402

1207 AGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTC 1266
403 S S S E T P N R H D M L L V E P L N R L 422

1267 CTGCAGGACAAGTGGGACAGATTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTAC 1326
423 L Q D K W D R F V K R I F Y F N F L V Y 442

1327 TGCCTGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCT 1386
443 C L Y M I I F T M A A Y Y R P V D G L P 462

1387 CCCTTTAAGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTG 1446
463 P F K M E K I G D Y F R V T G E I L S V 482

1447 TTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTCAGTATTTCCTGCAGAGGCGGCCGTCG 1506
483 L G G V Y F F F R G I Q Y F L Q R R P S 502

1507 ATGAAGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTC 1566
503 M K T L F V D S Y S E M L F F L Q S L F 522

1567 ATGCTGGCCACCGTGGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTA 1626
523 M L A T V V L Y F S H L K E Y V A S M V 542

1627 TTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATG 1686

FIG. 3CONT'D

543 F S L A L G W T N M L Y Y T R G F Q Q M 562

1687 GGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTT 1746
563 G I Y A V M I E K M I L R D L C R F M F 582

1747 GTCTACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGG 1806
583 V Y I V F L F G F S T A V V T L I E D G 602

1807 AAGAATGACTCCCTGCCGTCTGAGTCCACGTCGCACAGGTGGCGGGGGCCTGCCTGCAGG 1866
603 K N D S L P S E S T S H R W R G P A C R 622

1867 CCCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACC 1926
623 P P D S S Y N S L Y S T C L E L F K F T 642

1927 ATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCATC 1986
643 I G M G D L E F T E N Y D F K A V F I I 662

1987 CTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTC 2046
663 L L L A Y V I L T Y I L L L N M L I A L 682

2047 ATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGA 2106
683 M G E T V N K I A Q E S K N I W K L Q R 702

2107 GCCATCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGC 2166
703 A I T I L D T E K S F L K C M R K A F R 722

2167 TCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGC 2226
723 S G K L L Q V G Y T P D G K D D Y R W C 742

2227 TTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAA 2286
743 F R V D E V N W T T W N T N V G I I N E 762

2287 GACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCTGAGCTTCTCCCTGCGGTCAAGCAGA 2346
763 D P G N C E G V K R T L S F S L R S S R 782

2347 GTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCT 2406
783 V S G R H W K N F A L V P L L R E A S A 802

2407 CGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTG 2466
803 R D R Q S A Q P E E V Y L R Q F S G S L 822

2467 AAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGtgaggacgt 2526
823 K P E D A E V F K S P A A S G E K 839

2527 cacgcagacagcactgtcaacactgggccttaggagaccccgttgccacgggggctgct 2586

2587 gagggaacaccagtgctctgtcagcagcctggcctggtctgtgcctgcccagcatgttcc 2646

2647 caaatctgtgctggacaagctgtgggaagcgttcttggaagcatggggagtgatgtacat 2706

2707 ccaaccgtcactgtccccaagtgaatctcctaacagactttcaggttttactcacttta 2766

2767 ctaaacagtttggatggtcagtctctactgggacatgttaggcccttgttttctttgatt 2826

2827 ttattcttttctgtgagacagagttcactcttgttgcccaggctggagtgcagtggtgtg 2886

2887 atcttggctcactgcaacctctgctcccgggttcaagcgattcttctgcttcagtctccc 2946

FIG. 3CONT'D

2947 aagtagcttggattacaggtgagcactaccacgcccggctaatttttgtatttttaatag 3006

3007 agacggggtttcaccatgttggccaggctggtctcgaactcttgacctcaggtgatctgc 3066

3067 ccgccttggcctcccaaagtgctgggattacaggtgtgagccgctgcgctcggccttctt 3126

3127 tgattttatattattaggagcaaaagtaaatgaagcccaggaaaacacctttgggaacaa 3186

3187 actcttcctttgatggaaaatgcagaggcccttcctctctgtgccgtgcttgctcctctt 3246

3247 acctgcccgggtggtttgggggtgttggtgtttcctccctggagaagatgggggaggctg 3306

3307 tcccactcccagctctggcagaatcaagctgttgcagcagtgccttcttcatccttcctt 3366

3367 acgatcaatcacagtctccagaagatcagctcaattgctgtgcaggttaaaactacagaa 3426

3427 ccacatcccaaaggtacctggtaagaatgtttgaaagatcttccatttctaggaacccca 3486

3487 gtcctgcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataa 3546

3547 acagatatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 3591

FIG. 3CONT'D

FIG. 4

AMINO ACID SEQUENCE OF hVR1

```
  1  MKKWSSTDLG AAADPLQKDT CPDPLDGDPN SRPPPAKPQL STAKSRTRLF
 51  GKGDSEEAFP VDCPHEEGEL DSCPTITVSP VITIQRPGDG PTGARLLSQD
101  SVAASTEKTL RLYDRRSIFE AVAQNNCQDL ESLLLFLQKS KKHLTDNEFK
151  DPETGKTCLL KAMLNLHDGQ NTTIPLLLEI ARQTDSLKEL VNASYTDSYY
201  KGQTALHIAI ERRNMALVTL LVENGADVQA AAHGDFFKKT KGRPGFYFGE
251  LPLSLAACTN QLGIVKFLLQ NSWQTADISA RDSVGNTVLH ALVEVADNTA
301  DNTKFVTSMY NEILILGAKL HPTLKLEELT NKKGMTPLAL AAGTGKIGVL
351  AYILQREIQE PECRHLSRKF TEWAYGPVHS SLYDLSCIDT CEKNSVLEVI
401  AYSSSETPNR HDMLLVEPLN RLLQDKWDRF VKRIFYFNFL VYCLYMIIFT
451  MAAYYRPVDG LPPFKMEKIG DYFRVTGEIL SVLGGVYFFF RGIQYFLQRR
501  PSMKTLFVDS YSEMLFFLQS LFMLATVVLY FSHLKEYVAS MVFSLALGWT
551  NMLYYTRGFQ QMGIYAVMIE KMILRDLCRF MFVYIVFLFG FSTAVVTLIE
601  DGKNDSLPSE STSHRWRGPA CRPPDSSYNS LYSTCLELFK FTIGMGDLEF
651  TENYDFKAVF IILLLAYVIL TYILLLNMLI ALMGETVNKI AQESKNIWKL
701  QRAITILDTE KSFLKCMRKA FRSGKLLQVG YTPDGKDDYR WCFRVDEVNW
751  TTWNTNVGII NEDPGNCXGV KRTLSFSLRS SRVSGRHWKN FALVPLLREA
801  SARDRQSAQP EEVYLRQFSG SLKPEDAEVF KSPAASGEK*
```

Key

T/S predicted phosphorylation sites

Transmembrane domains

Ankyrin binding domains

FIG. 5

COMPARISON OF THE AMINO ACID SEQUENCE OF THE RAT (VR1) AND HUMAN (hVR1) VANILLOID PROTEINS.

```
                 10        20        30        40        50
VR1    MEQRASLDSEESESPPQENSCLDPPDRDPNCKPPPVKPHIFTTRSRTRLF
hVR1   MKKWSSTDLGAAADPLQKDTCPDPLDGDPNSRPPPAKPQLSTAKSRTRLF
                 60        70        80        90       100
VR1    GKGDSEEASPLDCPYEEGGLASCPIITVSSVLTIQRPGDGPASVRPSSQD
hVR1   GKGDSEEAFPVDCPHEEGELDSCPTITVSPVITIQRPGDGPTGARLLSQD
                110       120       130       140       150
VR1    SVSAG.EKPPRLYDRRSIFDAVAQSNCQELESLLPFLQRSKKRLTDSEFK
hVR1   SVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQKSKKHLTDNEFK
                160       170       180       190       200
VR1    DPETGKTCLLKAMLNLHNGQNDTIALLLDVARKTDSLKQFVNASYTDSYY
hVR1   DPETGKTCLLKAMLNLHDGQNTTIPLLLEIARQTDSLKELVNASYTDSYY
                210       220       230       240       250
VR1    KGQTALHIAIERRNMTLVTLLVENGADVQAAANGDFFKKTKGRPGFYFGE
hVR1   KGQTALHIAIERRNMALVTLLVENGADVQAAAHGDFFKKTKGRPGFYFGE
                260       270       280       290       300
VR1    LPLSLAACTNQLAIVKFLLQNSWQPADISARDSVGNTVLHALVEVADNTV
hVR1   LPLSLAACTNQLGIVKFLLQNSWQTADISARDSVGNTVLHALVEVADNTV
                310       320       330       340       350
VR1    DNTKFVTSMYNEILILGAKLHPTLKLEEITNRKGLTPLALAASSGKIGVL
hVR1   DNTKFVTSMYNEILILGAKLHPTLKLEELTNKKGMTPLALAAGTGKIGVL
                360       370       380       390       400
VR1    AYILQREIHEPECRHLSRKFTEWAYGPVHSSLYDLSCIDTCEKNSVLEVI
hVR1   AYILQREIQEPECRHLSRKFTEWAYGPVHSSLYDLSCIDTCEKNSVLEVI
                410       420       430       440       450
VR1    AYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFFVYCLYMIIFT
hVR1   AYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFLVYCLYMIIFT
                460       470       480       490       500
VR1    AAAYYRPVEGLPPYKLKWTVGDYFRVTGEILSVSGGVYFFFRGIQYFLQR
hVR1   MAAYYRPVDGLPPFKMEK.IGDYFRVTGEILSVLGGVYFFFRGIQYFLQR
                510       520       530       540       550
VR1    RPSLKSLFVDSYSEILFFVQSLFMLVSVVLYFSQRKEYVASMVFSLAMGW
hVR1   RPSMKTLFVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGW
                560       570       580       590       600
VR1    TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLI
hVR1   TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLI
                610       620       630       640       650
VR1    EDGKNNSLPMESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLE
hVR1   EDGKNDSLPSESTSHRWRGPACRPPDSSYNSLYSTCLELFKFTIGMGDLE
                660       670       680       690       700
VR1    FTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWK
hVR1   FTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWK
                710       720       730       740       750
VR1    LQRAITILDTEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVN
hVR1   LQRAITILDTEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCFRVDEVN
                760       770       780       790       800
VR1    WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSGRVSGRNWKNFALVPLLRD
hVR1   WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSSRVSGRHWKNFALVPLLRE
                810       820       830
VR1    ASTRDRHATQQEEVQLKHYTGSLKPEDAEVFKDSMVPGEK
hVR1   ASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEK
```

FIG. 6

FULL-LENGTH hRV1 CLONED INTO (A) pBLUESCRIPT SK(+) (hVR1pBSK) AND (B) pCIN5-NEW (hVR1pCIN5) VIA NotI/EcoRI RESTRICTION SITES.

6A 6B

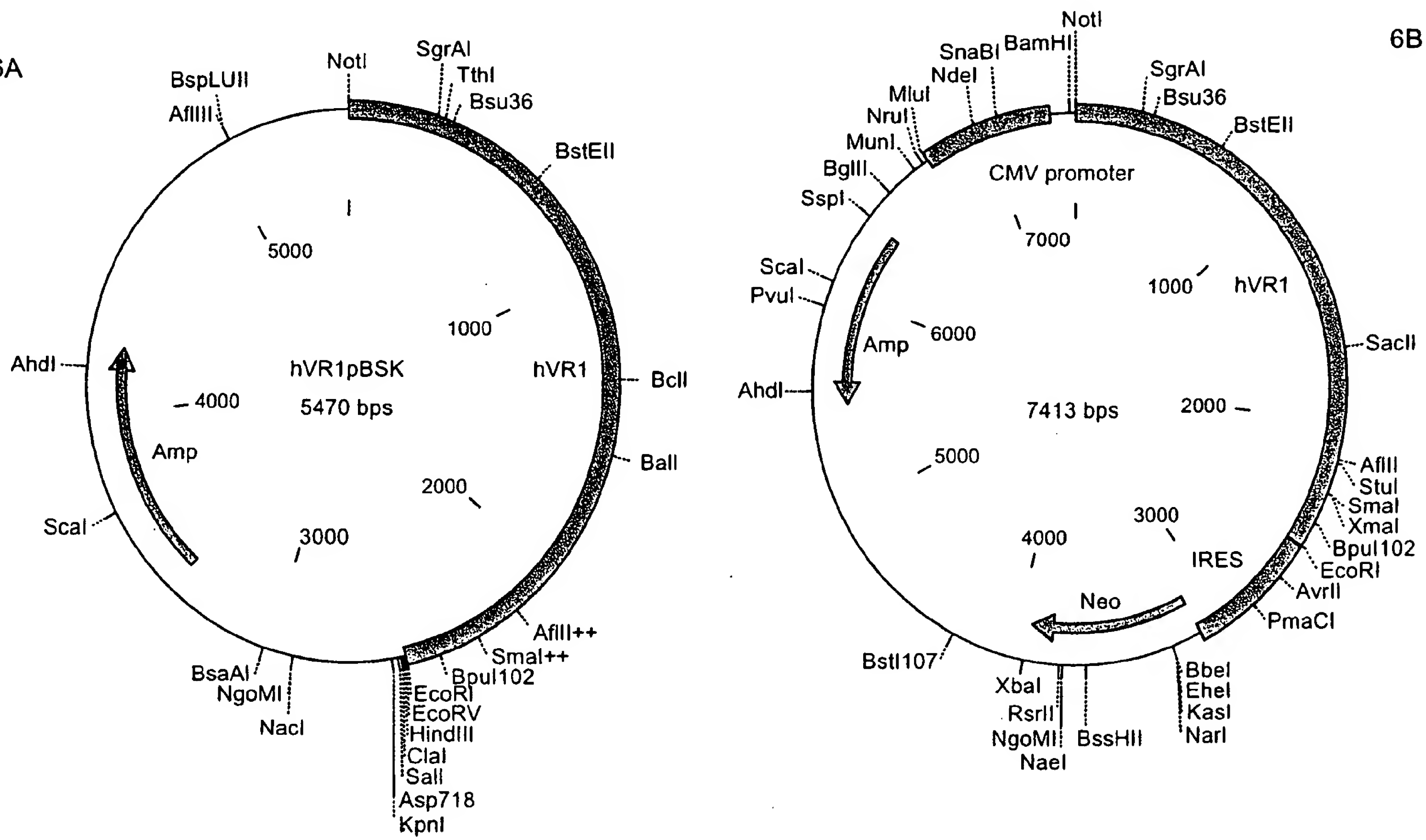
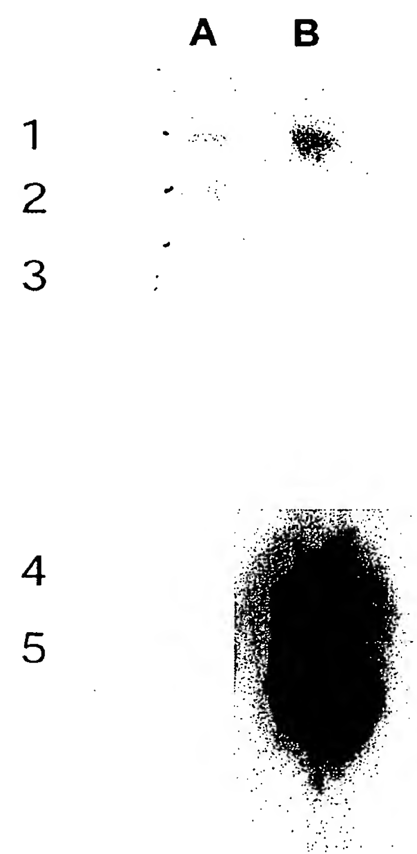


FIG. 7

SLOT HYBRIDISATION WITH hVR1 PROBE



Well
1A hDRG 1B hDRG
2A rDRG

3A Water

4B EST3 clone
5B 260bp Amplicon from Brain cDNA

FIG. 8

WESTERN BLOT PROBED WITH ANTI-hVR1 ANTIBODIES.
ARROW POINTS TO hVR1 SPECIFIC BAND

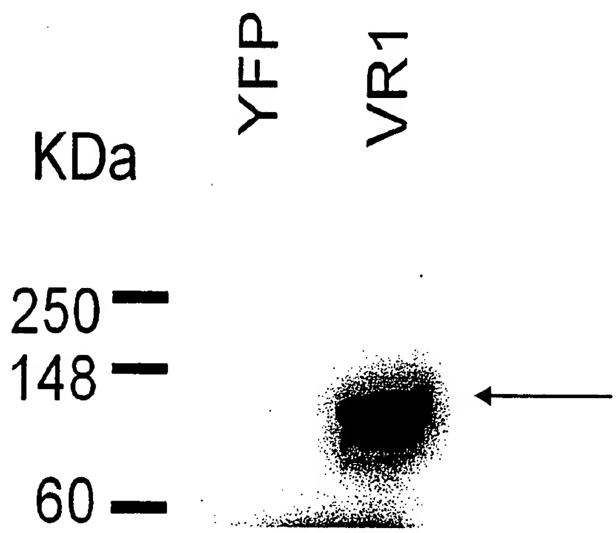


FIG. 9

IN SITU LOCALISATION OF VR1 IN RAT DRG TISSUE SECTIONS. ARROW POINTS TO A VR1 EXPRESSING SMALL DIAMETER (<25μn) NEURONE CELL BODY, MAGNIFICATION USED 147x10.

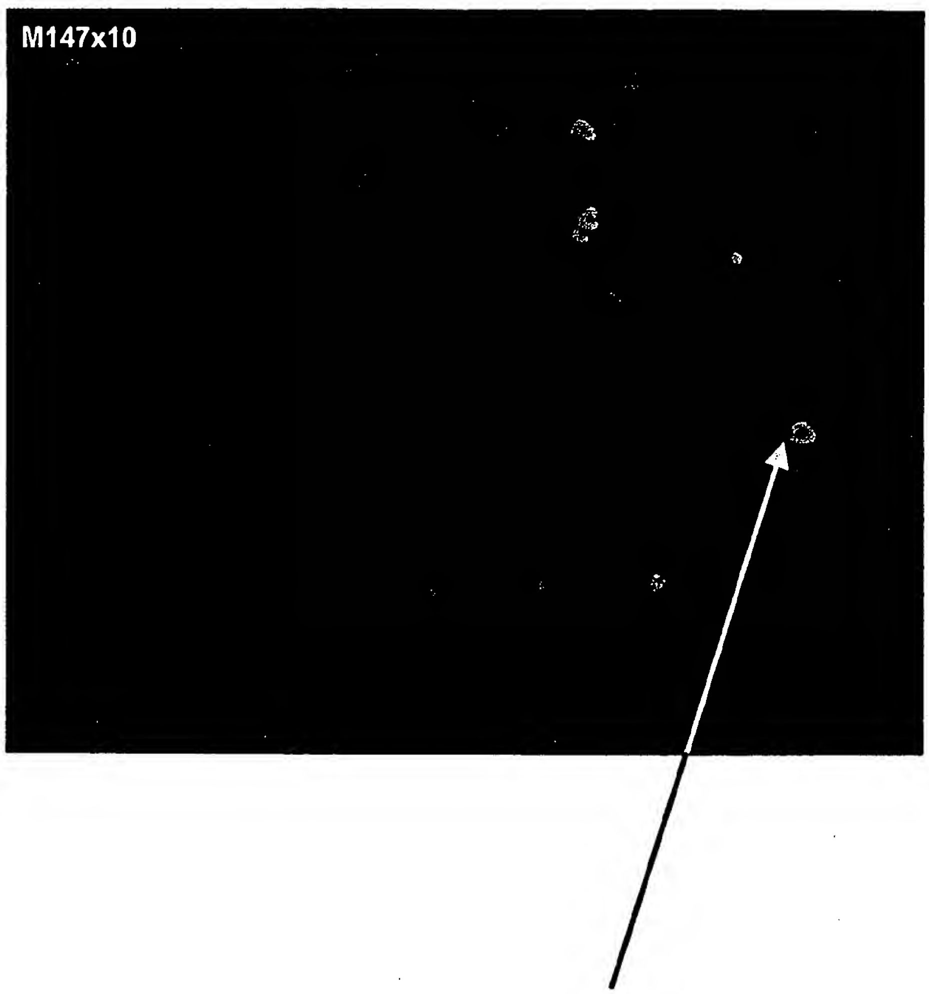
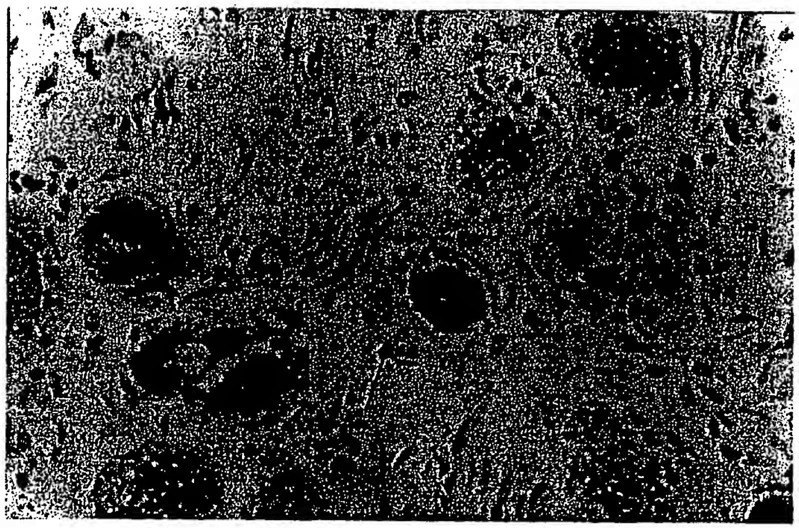


FIG. 10A



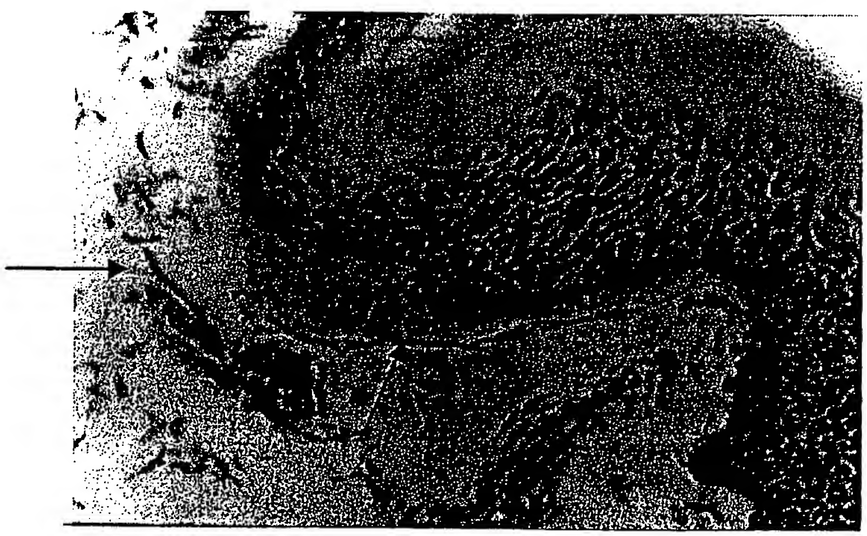


FIG. 10B

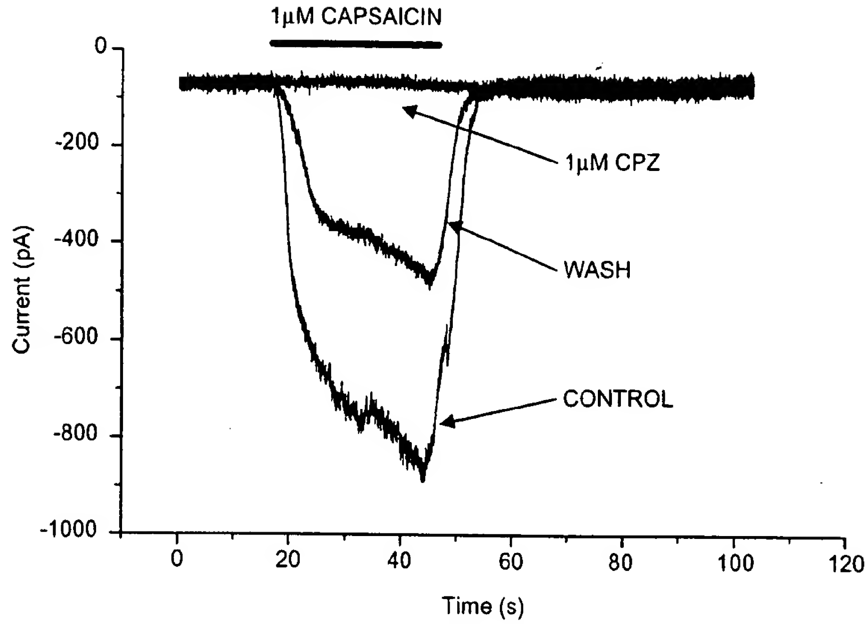
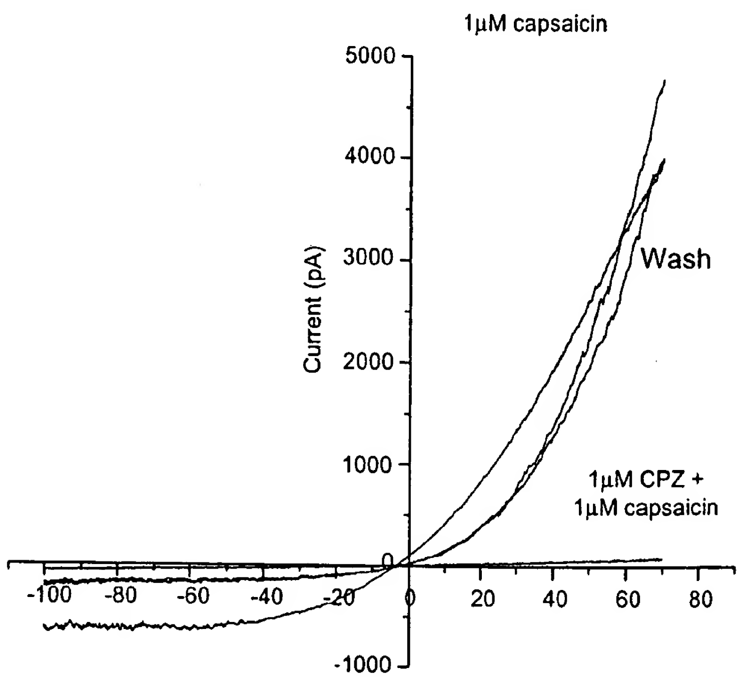


FIG. 11A



SOLUTIONS
OUTSIDE 140mM Na^+ 2mM Ca^{2+}
INSIDE 140mM Cs^+

FIG. 11B

FIG. 12A

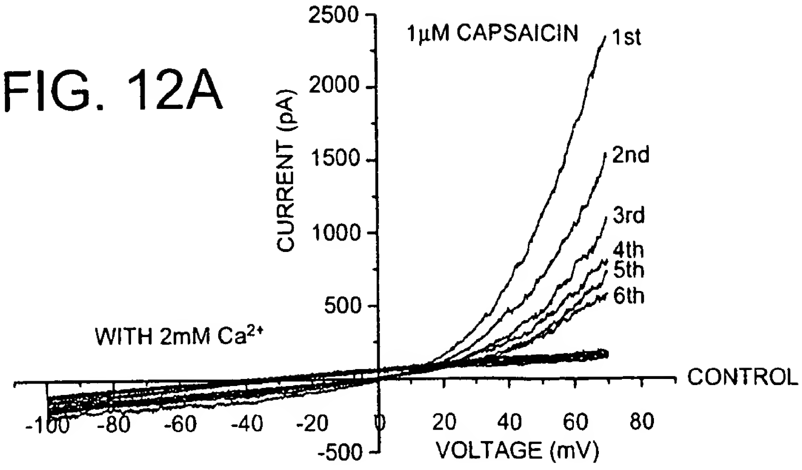


FIG. 12B

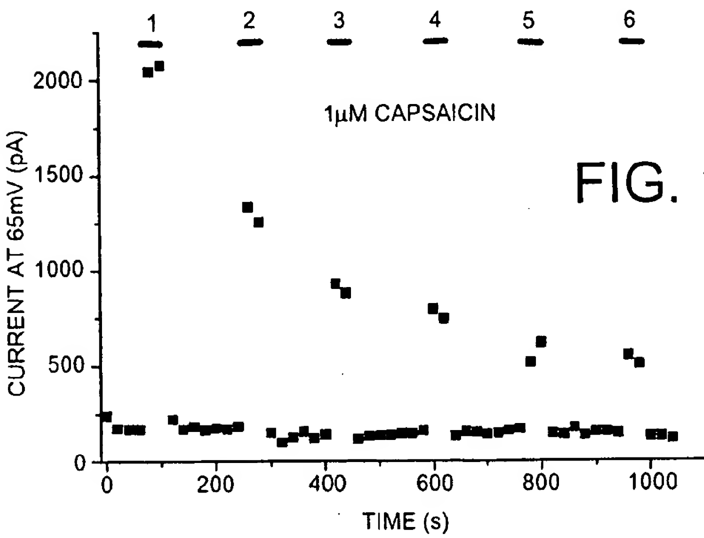
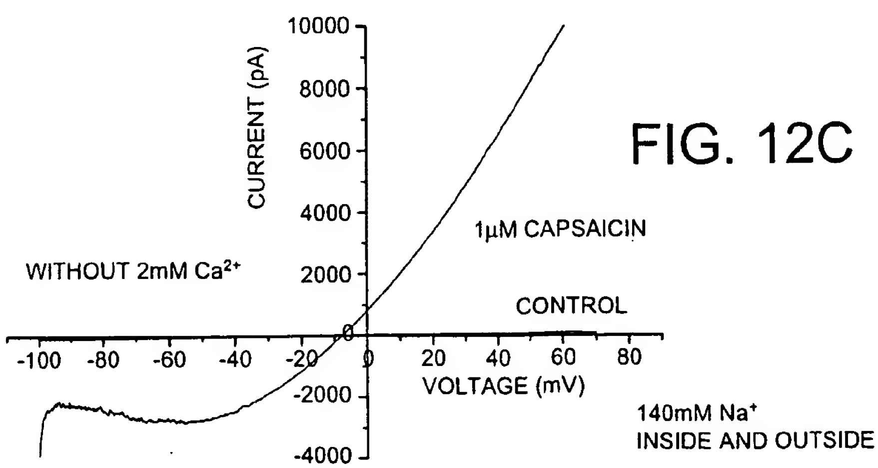
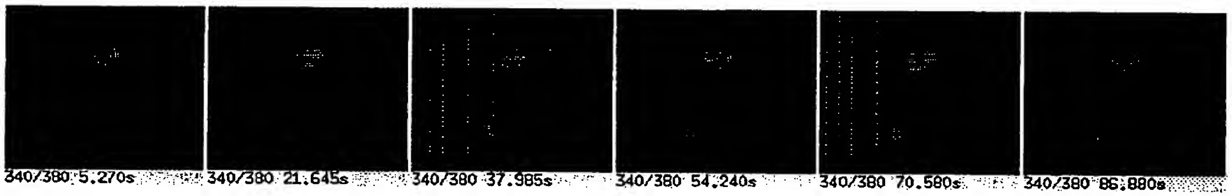


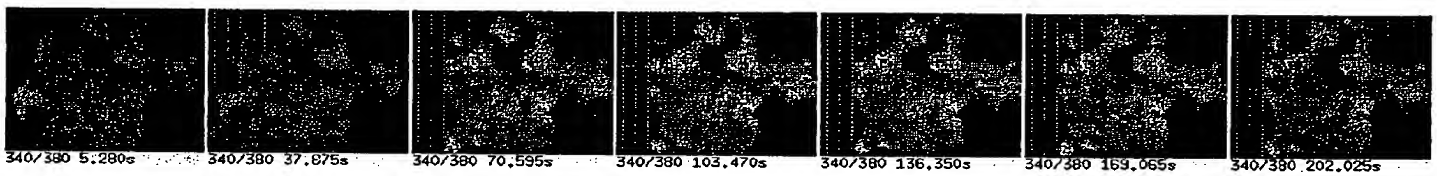
FIG. 12C



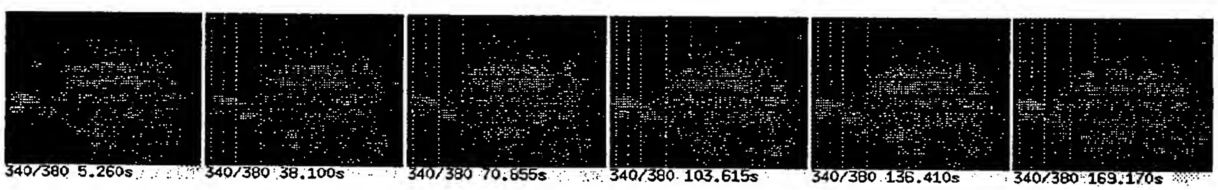
13A pCIN5-new in HEK293T, 24hr transient expression, stimulated with 3μM capsaicin at time point 52 secs of time course



13B hVR1pCIN5 in HEK293T, 24hr expression, stimulated with 1μM capsaicin at time point 52 seconds



13C hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation with 10 uM capsazepine, stimulated with 1 μM capsaicin at time point 52 seconds of time course



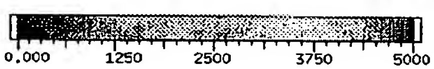
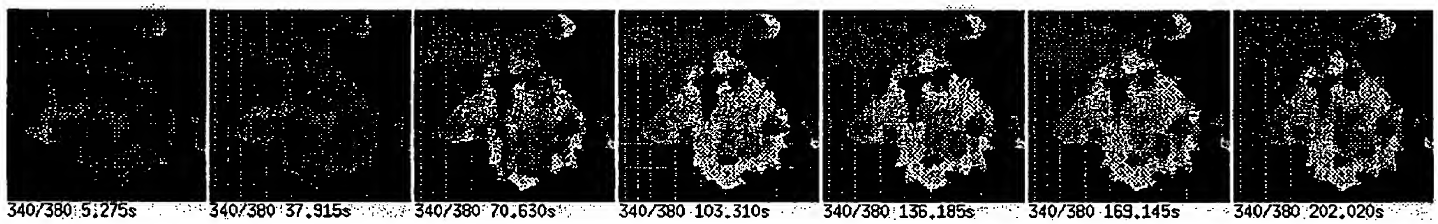
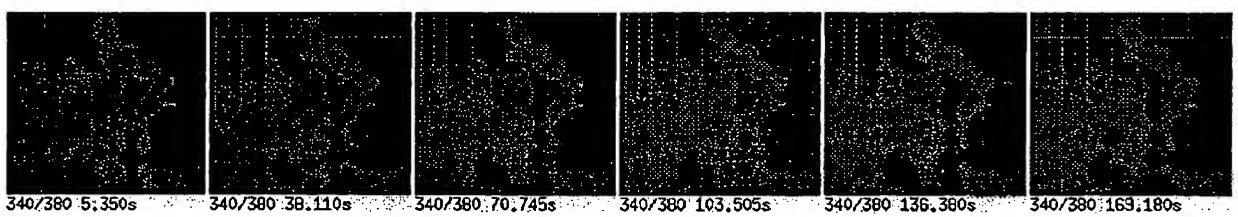


FIG. 13

13D hVR1pCIN5 in HEK293T, 24hr transient expression, stimulated with 10uM anandamide at time point 52 seconds



13E hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation in 10uM capsazepine, stimulated with 10uM anandamide at time point 52 sec



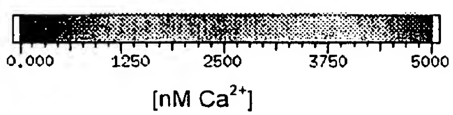
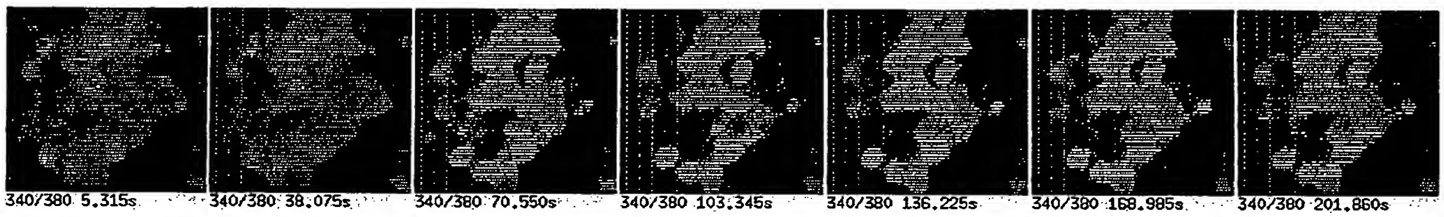


FIG. 13CONT'D

13F hVR1pCIN5 in HEK293T cells, 24hr transient expression, stimulated with 1uM Resiniferatoxin at time point 52 seconds



13G hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation with 10 uM capsazepine, stimulated with 1 uM Resiniferatoxin at time point 52 seconds

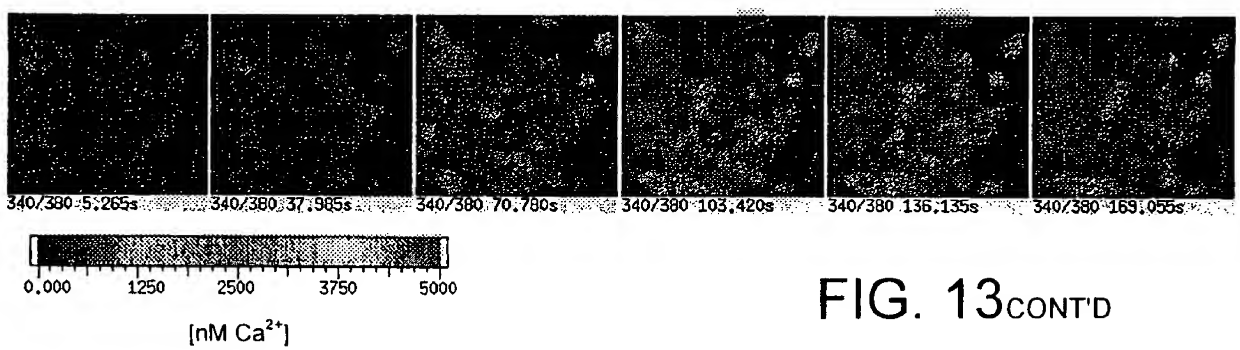
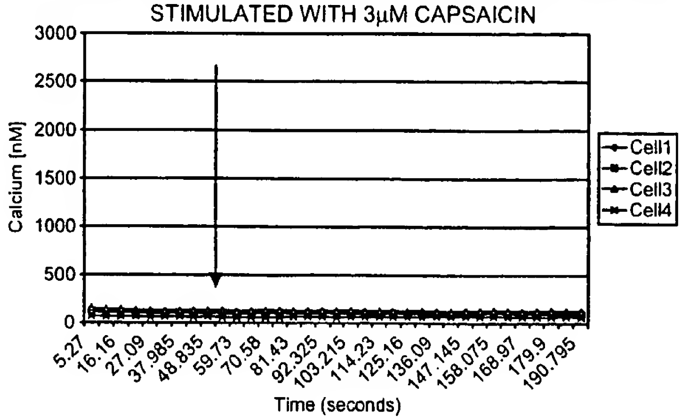


FIG. 13CONT'D

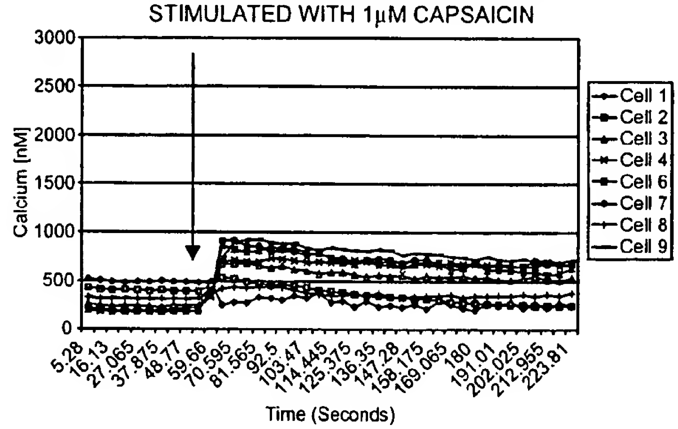
FIG. 14

EXPOSURE OF TRANSFECTED CELLS TO AGONISTS (ADDITION INDICATED BY ARROW).

14A: pCIN5-NEW IN HEK293T, 24hr TRANSIENT EXPRESSION, STIMULATED WITH 3μM CAPSAICIN



14B: hVR1pCIN5 IN HEK293T, 24hr EXPRESSION, STIMULATED WITH 1μM CAPSAICIN



14C: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT EXPRESSION, 20 MIN PRE-INCUBATION WITH 10μM CAPSAZEPINE, STIMULATION WITH 1μM CAPSIACIN

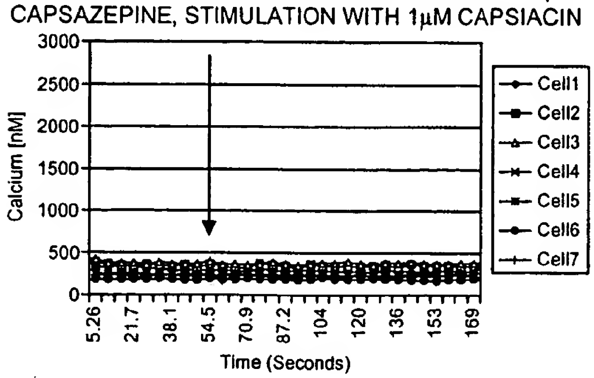
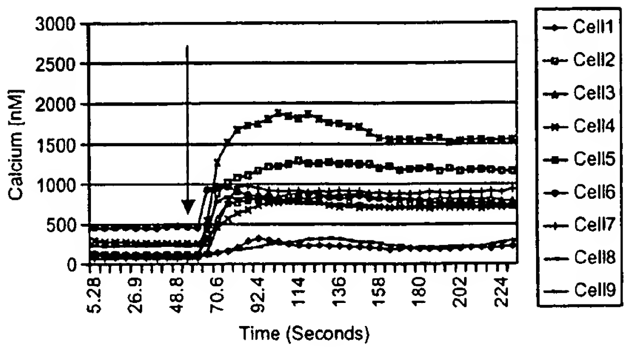


FIG. 14CONT'D

14D: hVR1pCIN5 IN HEK293T, 24hR TRANSIENT EXPRESSION, STIMULATION WITH 10μM ANANDAMIDE



14E: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT EXPRESSION, 20 MIN PRE-INCUBATION IN 10μM CAPAZEPINE, STIMULATED WITH 10μM ANANDAMIDE

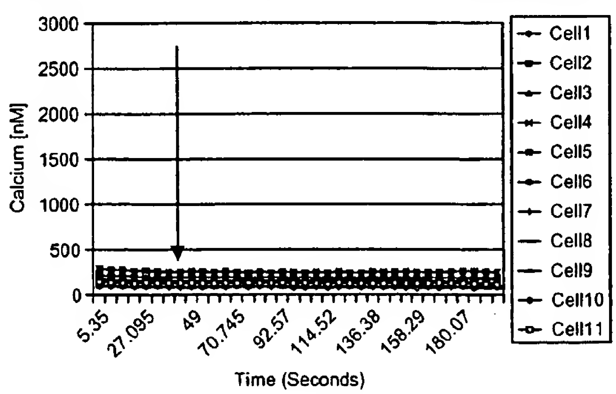
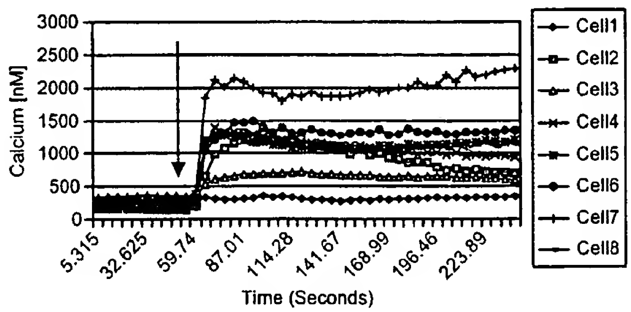
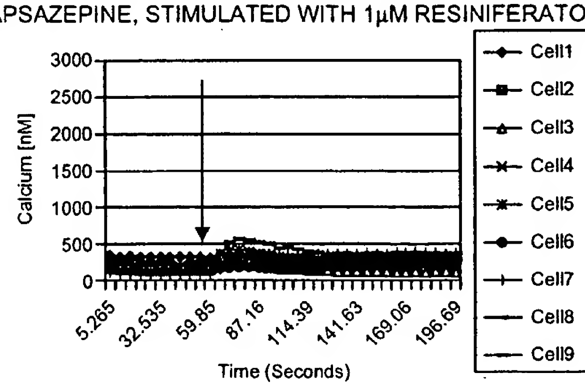


FIG. 14CONT'D

14F: hVR1pCIN5 IN HEK293T CELLS, 24hr TRANSIENT EXPRESSION, STIMULATED WITH 1μM RESINIFERATOXIN



14G: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT EXPRESSION, 20 MIN PRE-INCUBATION WITH 10μM CAPSAZEPINE, STIMULATED WITH 1μM RESINIFERATOXIN



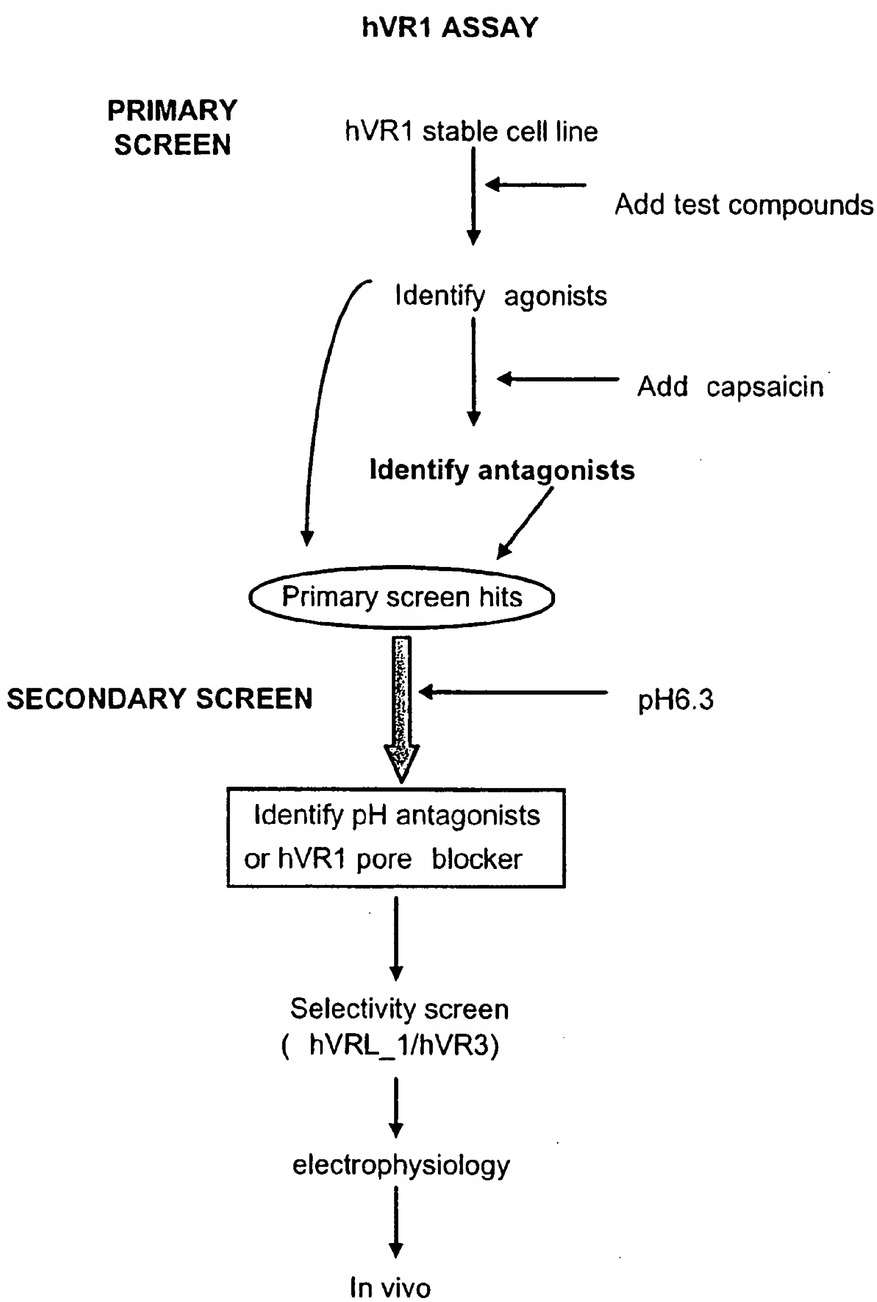

hVR1 ASSAY
PRIMARY SCREEN
hVR1 stable cell line
Add test compounds
Identify agonists
Add capsaicin
Identify antagonists
Primary screen hits
SECONDARY SCREEN
pH6.3
Identify pH antagonists or hVR1 pore blocker
Selectivity screen (hVRL_1/hVR3)
electrophysiology
In vivo

FIG. 15

FIG. 16

ALIGNMENT OF THE HUMAN VR3 *IN SILICO* CLUSTERS WITH RAT VR1

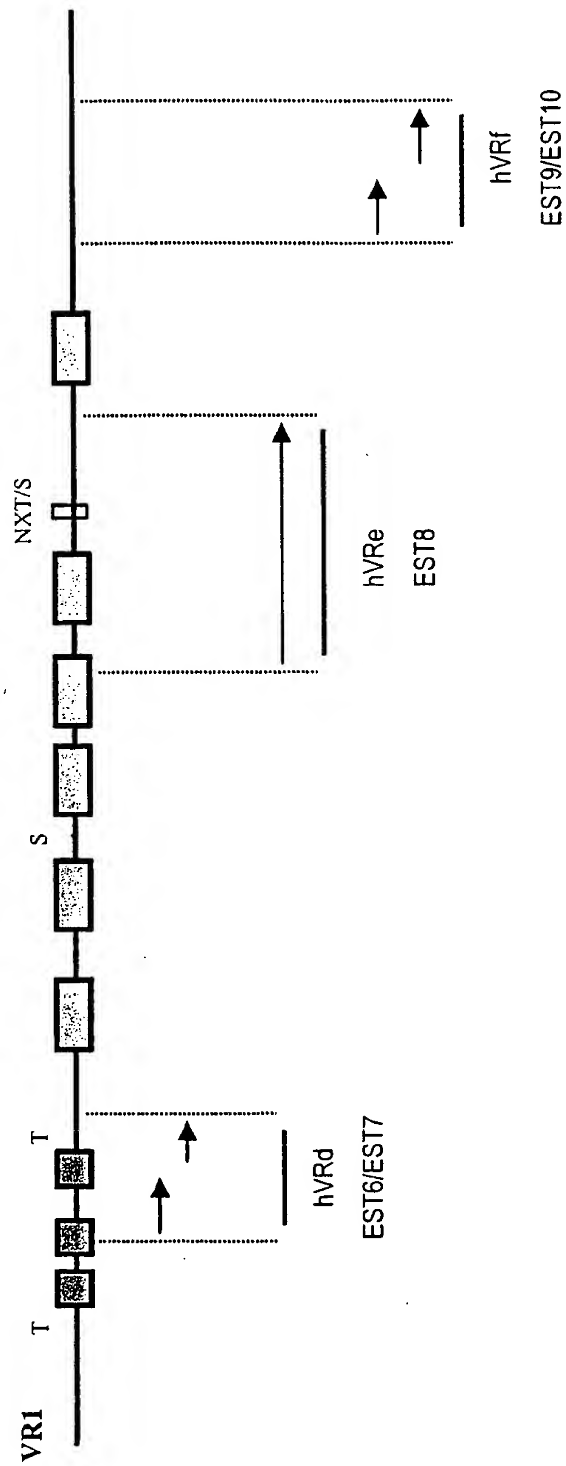


FIG. 17

hVR3 SEQUENCE INCLUDING 5' UTR (nt -686 TO nt 0) CODING REGION (nt1 TO nt 2889), 3'UTR (nt 2890 TO nt 3418)

```
-684  ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact  -625

-624  agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc  -565

-564  gagtgtgtgcaggccagggagggctttccagaggagcccagttgagctggaacaccagtg  -505

-504  gggaggagttgaccagcaaaggtgcagggagggatcagcactttgcactggggagcagag  -445

-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttcctgttctgtaaaatg  -385

-384  ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca  -325

-324  tggattttctgagcccctcctgtgcctgaagcccacggttaatggttctgccttagcagg  -265

-264  tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg  -205

-204  aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct  -145

-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggctctctggggctcca  -85

 -84  aagctggctttcttggtcagcagtagggtctgggatccaagtatggggtcccagcttgac  -25

 -24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG  35

  36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA  95

  96  CATCTGGGAGTGGCCTCCCTGTGCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC  155

 156  AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCGCCCCTGGCCGGGTT  215

 216  CTGCCTGACTCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG  275

 276  CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGAGGTGGCTGAGCTCCCCGG  335

 336  GGATGAGAGTGGCACCCCAGGTGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT  395
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396  TGAGGGGGAGGATGGCTCCCTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC  455
456  AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC  515
516  CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCAA  575
576  GAAAGCACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACCACTCCAGTGACAA  635
636  CAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGCAGAGCCCCAAAGCCCCTGCCCC  695
696  TCAGCCGCCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTGACATCGTGTCCCG  755
756  GGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTCTTGCTGACCCACAAGAAACGCCT  815
816  AACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGCCCAAGGCCTTGCT  875
876  GAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACATCGCGGAGCGCAC  935
936  CGGCAACATGCGGGAGTTCATTAACTCGCCCTTCCGTGACATCTACTATCGAGGTCAGAC  995
996  AGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAACTTCTCGTGGCCCA  1055
1056 GGGAGCTGATGTCCACGCCCAGGCCCGTGGGCGCTTCTTCCAGCCCAAGGATGAGGGGGG  1115
1116 CTACTTCTACTTTGGGGAGCTGCCCCTGTCGCTGGCTGCCTGCACCAACCAGCCCCACAT  1175
1176 TGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGCGCCAGGACTCGCG  1235
1236 AGGCAACACAGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACCCGTGAGAACACCAA  1295
1296 GTTTGTTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCGCCTCTTCCCCGACAG  1355
1356 CAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCTCATGATGGCTGCCAAGAC  1415
1416 GGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGGATGAGGACACACG  1475
1476 GCACCTGTCCCGCAAGTCCAAGGACTGGGCCTATGGGCCAGTGTATTCCTCGCTTTATGA  1535
```

FIG. 17CONT'D

1536 CCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCTCCGTGCTGGAGATCCTGGTGTACAA 1595

1596 CAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCAATGAACTGCTGCG 1655

1656 GGACAAGTGGCGGAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGGTCTCCTACCTGTG 1715

1716 TGCCATGGTTATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGGGCACACCGCCGTA 1775

1776 CCCTTACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTCATTACGCTCTTCAC 1835

1836 TGGGGTCCTGTTCTTCTTCACCAACATCAAAGACTTGTTCATGAAGAAATGCCCTGGAGT 1895

1896 GAATTCTCTCTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCTACTCTGTCCTGGT 1955

1956 GATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGGCCATGATGGTCTT 2015

2016 TGCCCTGGTCCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGCTGAAGCTGACGGG 2075

2076 GACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCCGATTCCTGCTCGT 2135

2136 CTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCCTGAACCCGTGTGC 2195

2196 CAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCACTTACCCCTCGTG 2255

2256 CCGTGACAGCGAGACCTTCAGCACCTTCCTCCTGGACCTGTTTAAGCTGACCATCGGCAT 2315

2316 GGGCGACCTGGAGATGCTGAGCAGCACCAAGTACCCCGTGGTCTTCATCATCCTGCTGGT 2375

2376 GACCTACATCATCCTCACCTCTGTGCTGCTCCTCAACATGCTCATTGCCCTCATGGGCGA 2435

2436 GACAGTGGGCCAGGTCTCCAAGGAGAGCAAGCACATCTGGAAGCTGCAGTGGGCCACCAC 2495

2496 CATCCTGGACATTGAGCGCTCCTTCCCCGTATTCCTGAGGAAGGCCTTCCGCTCTGGGGA 2555

2556 GATGGTCACCGTGGGCAAGAGCTCGGACGGCACTCCTGACCGCAGGTGGTGCTTCAGGGT 2615

2616 GGATGAGGTGAACTGGTCTCACTGGAACCAGAACTTGGGCATCATCAACGAGGACCCGGG 2675

FIG. 17 CONT'D

2676 CAAGAATGAGACCTACCAGTATTATGGCTTCTCGCATACCGTGGGCCGCCTCCGCAGGGA 2735

2736 TCGCTGGTCCTCGGTGGTACCCCGCGTGGTGGAACTGAACAAGAACTCGAACCCGGACGA 2795

2796 GGTGGTGGTGCCTCTGGACAGCATGGGGAACCCCCGCTGCGATGGCCACCAGCAGGGTTA 2855

2856 CCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCtagggactgcagcccagccccagctt 2915

2916 ctctgcccactcatttctagtccagccgcatttcagcagtgccttctggggtgtcccccc 2975

2976 acaccctgctttggccccagaggcgagggaccagtggaggtgccagggaggccccaggac 3035

3036 cctgtggtcccctggctctgcctccccaccctggggtgggggctcccggccacctgtctt 3095

3096 gctcctatggagtcacataagccaacgccagagcccctccacctcaggccccagcccctg 3155

3156 cctctccattatttatttgctctgctctcaggaagcgacgtgacccctgccccagctgga 3215

3216 acctggcagaggccttaggaccccgttccaagtgcactgcccggccaagccccagcctca 3275

3276 gcctgcgcctgagctgcatgcgccaccatttttggcagcgtggcagctttgcaaggggct 3335

3336 ggggccctcggcgtggggccatgccttctgtgtgttctgtagtgtctgggatttgccggt 3395

3396 gctcaataaatgtttattcattgaaaaaaaaaaaaaa 3433

FIG. 17CONT'D

FIG. 18

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR3 INCLUDING THE 5'UTR (nt -684 TO nt 0), CODING REGION (nt1 TO 2889) AND 3'UTR (nt 2890 TO nt 3418)

```
-684  ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact  -625

-624  agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc  -565

-564  gagtgtgtgcaggccagggagggctttccagaggagcccagttgagctggaacaccagtg  -505

-504  gggaggagttgaccagcaaaggtgcagggagggatcagcactttgcactggggagcagag  -445

-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttcctgttctgtaaaatg  -385

-384  ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca  -325

-324  tggattttctgagcccctcctgtgcctgaagcccacggttaatggttctgccttagcagg  -265

-264  tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg  -205

-204  aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct  -145

-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggctctctggggctcca  -85

 -84  aagctggctttcttggtcagcagtagggtctgggatccaagtatggggtcccagcttgac  -25

 -24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG  35
   1                           M  P  R  V  V  G  P  G  A  N  L  C   12

  36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA  95
  13   F  Q  V  R  E  R  G  S  C  C  S  S  R  L  R  L  A  A  N  H   32

  96  CATCTGGGAGTGGCCTCCCTGTGCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC  155
  33   I  W  E  W  P  P  C  A  P  V  I  T  T  V  A  L  K  Q  L  A   52

 156  AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCTGGCCGGGTT  215
  53   A  L  L  L  V  H  V  G  G  G  F  L  E  P  P  P  L  A  G  F   72

 216  CTGCCTGACTCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG  275
  73   C  L  T  P  L  S  F  P  C  R  L  S  S  A  D  G  P  G  A  G   92

 276  CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGGAGGTGGCTGAGCTCCCCGG  335
  93   M  A  D  S  S  E  G  P  R  A  G  P  G  E  V  A  E  L  P  G   112

 336  GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT  395
 113   D  E  S  G  T  P  G  G  E  A  F  P  L  S  S  L  A  N  L  F   132

 396  TGAGGGGGAGGATGGCTCCCTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC  455
 133   E  G  E  D  G  S  L  S  P  S  P  A  D  A  S  R  P  A  G  P   152

 456  AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC  515
 153   G  D  G  R  P  N  L  R  M  K  F  Q  G  A  F  R  K  G  V  P   172

 516  CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCAA  575
 173   N  P  I  D  L  L  E  S  T  L  Y  E  S  S  V  V  P  G  P  K   192
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```
 576 GAAAGCACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACCACTCCAGTGACAA  635
 193  K  A  P  M  D  S  L  F  D  Y  G  T  Y  R  H  H  S  S  D  N   212

 636 CAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGCAGAGCCCCAAAGCCCCTGCCCC  695
 213  K  R  W  R  K  K  I  I  E  K  Q  P  Q  S  P  K  A  P  A  P   232

 696 TCAGCCGCCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTGACATCGTGTCCCG  755
 233  Q  P  P  P  I  L  K  V  F  N  R  P  I  L  F  D  I  V  S  R   252

 756 GGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTCTTGCTGACCCACAAGAAACGCCT  815
 253  G  S  T  A  D  L  D  G  L  L  P  F  L  L  T  H  K  K  R  L   272

 816 AACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGCCCAAGGCCTTGCT  875
 273  T  D  E  E  F  R  E  P  S  T  G  K  T  C  L  P  K  A  L  L   292

 876 GAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACATCGCGGAGCGCAC  935
 293  N  L  S  N  G  R  N  D  T  I  P  V  L  L  D  I  A  E  R  T   312

 936 CGGCAACATGCGGGAGTTCATTAACTCGCCCTTCCGTGACATCTACTATCGAGGTCAGAC  995
 313  G  N  M  R  E  F  I  N  S  P  F  R  D  I  Y  Y  R  G  Q  T   332

 996 AGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAACTTCTCGTGGCCCA  1055
 333  A  L  H  I  A  I  E  R  R  C  K  H  Y  V  E  L  L  V  A  Q   352

1056 GGGAGCTGATGTCCACGCCCAGGCCCGTGGGCGCTTCTTCCAGCCCAAGGATGAGGGGGG  1115
 353  G  A  D  V  H  A  Q  A  R  G  R  F  F  Q  P  K  D  E  G  G   372

1116 CTACTTCTACTTTGGGGAGCTGCCCCTGTCGCTGGCTGCCTGCACCAACCAGCCCCACAT  1175
 373  Y  F  Y  F  G  E  L  P  L  S  L  A  A  C  T  N  Q  P  H  I   392

1176 TGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGCGCCAGGACTCGCG  1235
 393  V  N  Y  L  T  E  N  P  H  K  K  A  D  M  R  R  Q  D  S  R   412

1236 AGGCAACACAGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACCCGTGAGAACACCAA  1295
 413  G  N  T  V  L  H  A  L  V  A  I  A  D  N  T  R  E  N  T  K   432

1296 GTTTGTTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCGCCTCTTCCCCGACAG  1355
 433  F  V  T  K  M  Y  D  L  L  L  L  K  C  A  R  L  F  P  D  S   452

1356 CAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCTCATGATGGCTGCCAAGAC  1415
 453  N  L  E  A  V  L  N  N  D  G  L  S  P  L  M  M  A  A  K  T   472

1416 GGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGGATGAGGACACACG  1475
 473  G  K  I  G  I  F  Q  H  I  I  R  R  E  V  T  D  E  D  T  R   492

1476 GCACCTGTCCCGCAAGTCCAAGGACTGGGCCTATGGGCCAGTGTATTCCTCGCTTTATGA  1535
 493  H  L  S  R  K  S  K  D  W  A  Y  G  P  V  Y  S  S  L  Y  D   512

1536 CCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCTCCGTGCTGGAGATCCTGGTGTACAA  1595
 513  L  S  S  L  D  T  C  G  E  E  A  S  V  L  E  I  L  V  Y  N   532

1596 CAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCAATGAACTGCTGCG  1655
 533  S  K  I  E  N  R  H  E  M  L  A  V  E  P  I  N  E  L  L  R   552

1656 GGACAAGTGGCGGAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGGTCTCCTACCTGTG  1715
 553  D  K  W  R  K  F  G  A  V  S  F  Y  I  N  V  V  S  Y  L  C   572
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FIG. 18CONT'D

```
1716 TGCCATGGTTATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGGGCACACCGCCGTA 1775
573   A  M  V  I  F  T  L  T  A  Y  Y  Q  P  L  E  G  T  P  P  Y  592

1776 CCCTTACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTCATTACGCTCTTCAC 1835
593   P  Y  R  T  T  V  D  Y  L  R  L  A  G  E  V  I  T  L  F  T  612

1836 TGGGGTCCTGTTCTTCTTCACCAACATCAAAGACTTGTTCATGAAGAAATGCCCTGGAGT 1895
613   G  V  L  F  F  F  T  N  I  K  D  L  F  M  K  K  C  P  G  V  632

1896 GAATTCTCTCTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCTACTCTGTCCTGGT 1955
633   N  S  L  F  I  D  G  S  F  Q  L  L  Y  F  I  Y  S  V  L  V  652

1956 GATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGGCCATGATGGTCTT 2015
653   I  V  S  A  A  L  Y  L  A  G  I  E  A  Y  L  A  M  M  V  F  672

2016 TGCCCTGGTCCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGCTGAAGCTGACGGG 2075
673   A  L  V  L  G  W  M  N  A  L  Y  F  T  R  G  L  K  L  T  G  692

2076 GACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCCGATTCCTGCTCGT 2135
693   T  Y  S  I  M  I  Q  K  I  L  F  K  D  L  F  R  F  L  L  V  712

2136 CTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCCTGAACCCGTGTGC 2195
713   Y  L  L  F  M  I  G  Y  A  S  A  L  V  S  L  L  N  P  C  A  732

2196 CAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCACTTACCCCTCGTG 2255
733   N  M  K  V  C  N  E  D  Q  T  N  C  T  V  P  T  Y  P  S  C  752

2256 CCGTGACAGCGAGACCTTCAGCACCTTCCTCCTGGACCTGTTTAAGCTGACCATCGGCAT 2315
753   R  D  S  E  T  F  S  T  F  L  L  D  L  F  K  L  T  I  G  M  772

2316 GGGCGACCTGGAGATGCTGAGCAGCACCAAGTACCCCGTGGTCTTCATCATCCTGCTGGT 2375
773   G  D  L  E  M  L  S  S  T  K  Y  P  V  V  F  I  I  L  L  V  792

2376 GACCTACATCATCCTCACCTCTGTGCTGCTCCTCAACATGCTCATTGCCCTCATGGGCGA 2435
```

```
2856  CCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCtagggactgcagcccagccccagctt  2915
 953   P  R  K  W  R  T  D  D  A  P  L                              963

2916  ctctgcccactcatttctagtccagccgcatttcagcagtgccttctggggtgtcccccc  2975

2976  acaccctgctttggccccagaggcgagggaccagtggaggtgccagggaggccccaggac  3035

3036  cctgtggtcccctggctctgcctccccaccctggggtgggggctcccggccacctgtctt  3095

3096  gctcctatggagtcacataagccaacgccagagcccctccacctcaggccccagcccctg  3155

3156  cctctccattatttatttgctctgctctcaggaagcgacgtgacccctgccccagctgga  3215

3216  acctggcagaggccttaggaccccgttccaagtgcactgcccggccaagccccagcctca  3275

3276  gcctgcgcctgagctgcatgcgccaccattttggcagcgtggcagctttgcaaggggct  3335

3336  ggggccctcggcgtggggccatgccttctgtgtgttctgtagtgtctgggatttgccggt  3395

3396  gctcaataaatgtttattcattgaaaaaaaaaaaaaaa  3433
```

FIG. 18CONT'D

FIG. 19

AMINO ACID SEQUENCE OF hVR3

1 MPRVVGPGAN LCFQVRERGS CCSSRLRLAA NHIWEWPPCA PVITTVALKQ
51 LAALLLVHVG GGFLEPPPLA GFCLTPLSFP CRLSSADGPG AGMADSSEGP
101 RAGPGEVAEL PGDESGTPGG EAFPLSSLAN LFEGEDGSLS PSPADASRPA
151 GPGDGRPNLR MKFQGAFRKG VPNPIDLLES TLYESSVVPG PKKAPMDSLF
201 DYGTYRHHSS DNKRWRKKII EKQPQSPKAP APQPPPILKV FNRPILFDIV
251 SRGSTADLDG LLPFLLTHKK RLTDEEFREP STGKTCLPKA LLNLSNGRND
301 TIPVLLDIAE RTGNMREFIN SPFRDIYYRG QTALHIAIER RCKHYVELLV
351 AQGADVHAQA RGRFFQPKDE GGYFYFGELP LSLAACTNQP HIVNYLTENP
401 HKKADMRRQD SRGNTVLHAL VAIADNTREN TKFVTKMYDL LLLKCARLFP
451 DSNLEAVLNN DGLSPLMMAA KTGKIGIFQH IIRREVTDED TRHLSRKSKD
501 WAYGPVYSSL YDLSSLDTCG EEASVLEILV YNSKIENRHE MLAVEPINEL
551 LRDKWRKFGA VSFYINVVSY LCAMVIFTLT AYYQPLEGTP PYPYRTTVDY
601 LRLAGEVITL FTGVLFFFTN IKDLFMKKCP GVNSLFIDGS FQLLYFIYSV
651 LVIVSAALYL AGIEAYLAMM VFALVLGWMN ALYFTRGLKL TGTYSIMIQK
701 ILFKDLFRFL LVYLLFMIGY ASALVSLLNP CANMKVCNED QTNCTVPTYP
751 SCRDSETFST FLLDLFKLTI GMGDLEMLSS TKYPVVFIIL LVTYIILTSV
801 LLLNMLIALM GETVGQVSKE SKHIWKLQWA TTILDIERSF PVFLRKAFRS
851 GEMVTVGKSS DGTPDRRWCF RVDEVNWSHW NQNLGIINED PGKNETYQYY
901 GFSHTVGRLR RDRWSSVVPR VVELNKNSNP DEVVVPLDSM GNPRCDGHQQ
951 GYPRKWRTDD APL

Key

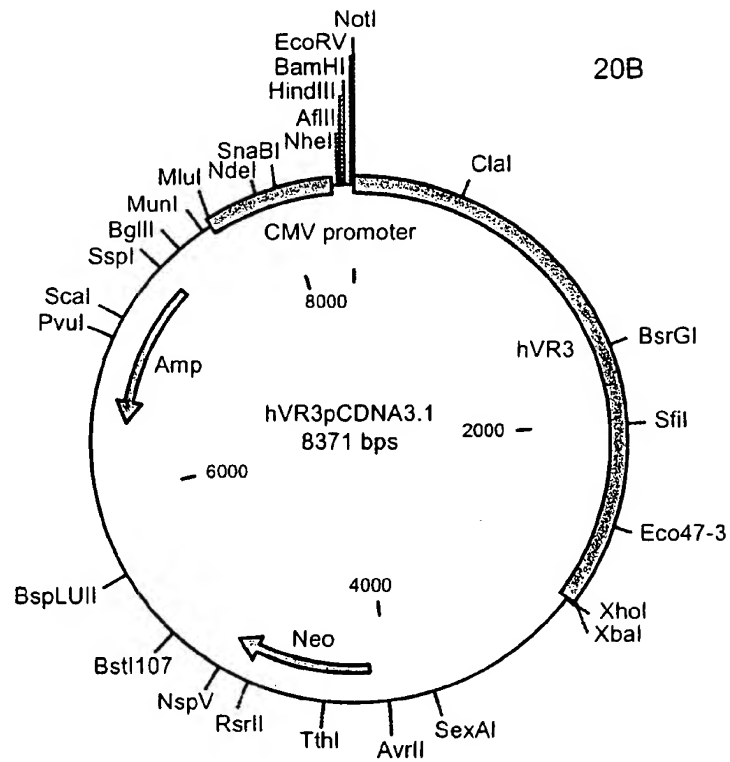
Transmembrane domains

Ankyrin binding domains

FIG. 20

ՎTO (A) pBLUESCRIPT SK(+) (hVR3pBSK) AND
NA3.1) VIA NotI/XhoI RESTRICTION SITES.

20B



BI

GI

FIG. 21

A MULTIPLE COMPARISON OF THE AMINO ACID SEQUENCES OF THE RAT VR1 AND THE HUMAN VANILLOID RECEPTORS, hVR1, hVRL-1 AND hRV3

```
                   10        20        30        40        50
VR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR1    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVRL-1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3    MPRVVGPGANLCFQVRERGSCCSSRLRLAANHIWEWPPCAPVITTVALKQ

                   60        70        80        90       100
VR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR1    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVRL-1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3    LAALLLVHVGGGFLEPPPLAGFCLTPLSFPCRLSSADGPGAGMADSSEGP

                  110       120       130       140       150
VR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~MEQRASLDSEESESPPQENSCL
hVR1    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~MKKWSSTDLGAAADPLQKDTCP
hVRL-1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3    RAGPGEVAELPGDESGTPGGEAFPLSSLANLFEGEDGSLSPSPADASRPA

                  160       170       180       190       200
VR1     DPPDRDPNCKPPPVKPHIFTTRSRTRLFG....KGDSEEASPLDCPYEEG
hVR1    DPLDGDPNSRPPPAKPQLSTAKSRTRLFG....KGDSEEAFPVDCPHEEG
hVRL-1  ~~~~~~~~~~~~~~~~~~MTSPSSSPVFRLETLDGGQEDGSEADRGKLDF
hVR3    GPGDGRPNLRMKFQGAFRKGVPNP.....IDLLESTLYESSVVPGPKKAP

                  210       220       230       240       250
VR1     GLASCPIITVSSVLTIQRPGDGPASVRPSSQDSVSAG.EKP.PRLYDRRS
hVR1    ELDSCPTITVSPVITIQRPGDGPTGARLLSQDSVAASTEKT.LRLYDRRS
hVRL-1  GSGLPPM..ESQFQGEDRKFAPQIRVNLNYRKGTGASQPDP.NR.FDRDR
hVR3    MDSLFDYGTYRHHSSDNKRWRKKIIEKQPQSPKAPAPQPPPILKVFNRPI

                  260       270       280       290       300
VR1     IFDAVAQSNCQELESLLPFLQRSKKRLTDSEFKDPETGKTCLLKAMLNLH
hVR1    IFEAVAQNNCQDLESLLLFLQKSKKHLTDNEFKDPETGKTCLLKAMLNLH
hVRL-1  LENAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCLMKAVLNLK
hVR3    LFDIVSRGSTADLDGLLPFLLTHKKRLTDEEFREPSTGKTCLPKALLNLS

                  310       320       330       340       350
VR1     NGQNDTIALLLDVARKTDSLKQFVNASYTDSYYKGQTALHIAIERRNMTL
hVR1    DGQNTTIPLLLEIARQTDSLKELVNASYTDSYYKGQTALHIAIERRNMAL
hVRL-1  DGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQC
hVR3    NGRNDTIPVLLDIAERTGNMREFINSPFRDIYYRGQTALHIAIERRCKHY

                  360       370       380       390       400
VR1     VTLLVENGADVQAAANGDFFKKTKGRPGFYFGELPLSLAACTNQLAIVKF
hVR1    VTLLVENGADVQAAAHGDFFKKTKGRPGFYFGELPLSLAACTNQLGIVKF
hVRL-1  VKLLVENGANVHARACGRFFQKGQG.TCFYFGELPLSLAACTKQWDVVSY
hVR3    VELLVAQGADVHAQARGRFFQPKDEGGYFYFGELPLSLAACTNQPHIVNY

                  410       420       430       440       450
VR1     LLQNSWQPADISARDSVGNTVLHALVEVADNTVDNTKFVTSMYNEILILG
hVR1    LLQNSWQTADISARDSVGNTVLHALVEVADNTADNTKFVTSMYNEILILG
hVRL-1  LLENPHQPASLQATDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAG
hVR3    LTENPHKKADMRRQDSRGNTVLHALVAIADNTRENTKFVTKMYDLLLLKC

                  460       470       480       490       500
VR1     AKLHPTLKLEEITNRKGLTPLALAASSGKIGVLAYILQREIHEPECRHLS
hVR1    AKLHPTLKLEELTNKKGMTPLALAAGTGKIGVLAYILQREIQEPECRHLS
hVRL-1  ARLCPTVQLEEDIRNLQDLTPLKLAAKEGKIEIFRHILQREFS..GLSHLS
hVR3    ARLFPDSNLEAVLNNDGLSPLMMAAKTGKIGIFQHIIRREVTDEDTRHLS
```

```
                510       520       530       540       550
VR1     RKFTEWAYGPVHSSLYDLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLV
hVR1    RKFTEWAYGPVHSSLYDLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLV
hVRL-1  RKFTEWCYGPVRVSLYDLASVDSC.EENSVLEIIAF.HCKSPHRHRMVVL
hVR3    RKSKDWAYGPVYSSLYDLSSLDTCGEEASVLEILVY.WSKIENRHEMLAV

                560       570       580       590       600
VR1     EPLNRLLQDKWDRFVKRIFYFNFFVYCLYMIIFTAAAYYRPV..EGLPPY
hVR1    EPLNRLLQDKWDRFVKRIFYFNFLVYCLYMIIFTMAAYYRPV..DGLPPF
hVRL-1  EPLNKLLQAKWDLLIPK.FFLNFLCNLIYMFIFTAVAYHQPTLKKQAAPH
hVR3    EPINELLRDKWRKFGAVSFYINVVSYLCAMVIFTLTAYYQPL..EGTPPY

                610       620       630       640       650
VR1     KLKNTVGDYFRVTGEILSVSGGVYFFFRGIQ.YFLQRRPSLKSLFVDSYS
hVR1    KMEN.IGDYFRVTGEILSVLGGVYFFFRGIQ.YFLQRRPSMKTLFVDSYS
hVRL-1  .LNAEVGNSMLLTGHILILLGGIYLLVGQLW.YFWRRHVFIWISFIDSYF
hVR3    PYRTTV.DYLRLAGEVITLFTGVLFFFTNIKDLFMKKCPGVNSLFIDGSF

                660       670       680       690       700
VR1     EILFFVQSLFMLVSVVLYFSQRKEYVASMVFSLAMGWTNMLYYTRGFQQM
hVR1    EMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGWTNMLYYTRGFQQM
hVRL-1  EILFLFQALLTVVSQVLCFLAIEWYLPLLVSALVLGWLNLLYYTRGFQHT
hVR3    QLLYFIYSVLVIVSAALYLAGIEAYLAMMVFALVLGWMNALYFTRGLKLT

                710       720       730       740       750
VR1     GIYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLIEDGKNNSLP....
hVR1    GIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLIEDGKNDSLP....
hVRL-1  GIYSVMIQKVILRDLLRFLLIYLVFLFGFAVALVSLSQEAWRPEAPTGPN
hVR3    GTYSIMIQKILFKDLFRFLLVYLLFMIGYASALVSLLNPCANMKVCNEDQ

                760       770       780       790       800
VR1     MESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLEFTENYDFKA
hVR1    SESTSHRWRGPACRPPDSSYNSLYSTCLELFKFTIGMGDLEFTENYDFKA
hVRL-1  ATESVQPMEGQEDEGNGAQYRGILEASLELFKFTIGMGELAFQEQLHFRG
hVR3    TNCTVPTY..PSCR.DSETFSTFL...LDLFKLTIGMGDLEMLSSTKYPV

                810       820       830       840       850
VR1     VFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWKLQRAITILD
hVR1    VFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWKLQRAITILD
hVRL-1  MVLLLLLAYVLLTYILLLNMLIALMSETVNSVATDSWSIWKLQKAISVLE
hVR3    VFIILLVTYIILTSVLLLNMLIALMGETVGQVSKESKHIWKLQWATTILD

                860       870       880       890       900
VR1     TEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVNWTTWNTNVG
hVR1    TEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCFRVDEVNWTTWNTNVG
hVRL-1  MENGYWWC.RKKQRAGVMLTVGTKPDGSPDERWCFRVEEVNWASWEQTLP
hVR3    IERSFPVFLRKAFRSGEMVTVGKSSDGTPDRRWCFRVDEVNWSHWNQNLG

                910       920       930       940       950
VR1     IINEDPGNCE.....GVKRTLSFSLRSG.........RVSGRNWKNFALV
hVR1    IINEDPGNCE.....GVKRTLSFSLRSS.........RVSGRHWKNFALV
hVRL-1  TLCEDPSGA......GVPRTLENPVLAS.....PPKEDEDGASEENYVPV
hVR3    IINEDPGKWETYQYYGFSHTVGRLRRDRWSSVVPRVVELNKNSNPDEVVV

                960       970       980       990
VR1     PLLRDASTRDRHATQQEEVQLKHYTGSLKPEDAEVFKDSMVPGEN
hVR1    PLLREASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEN
hVRL-1  QLLQSN~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3    PLDSMGNPRCDGHQQGYPRKWRTDDAPL~~~~~~~~~~~~~~~~~
```

FIG. 21 CONT'D

FIG. 22A

HYBRIDISATION OF A NORTHERN BLOT WITH hVR3

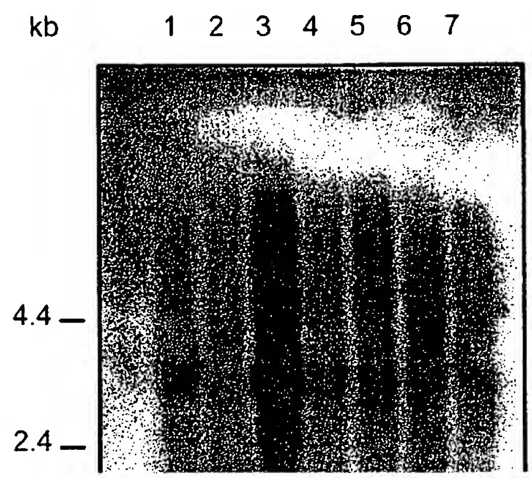
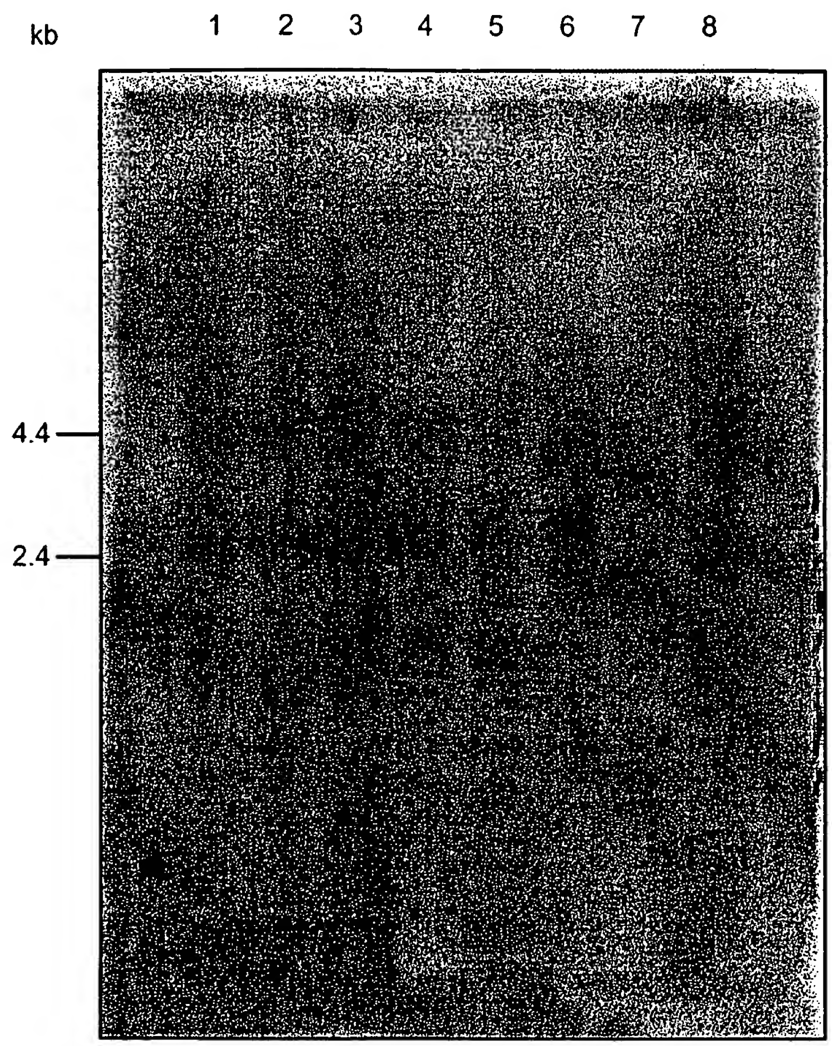


FIG. 22B

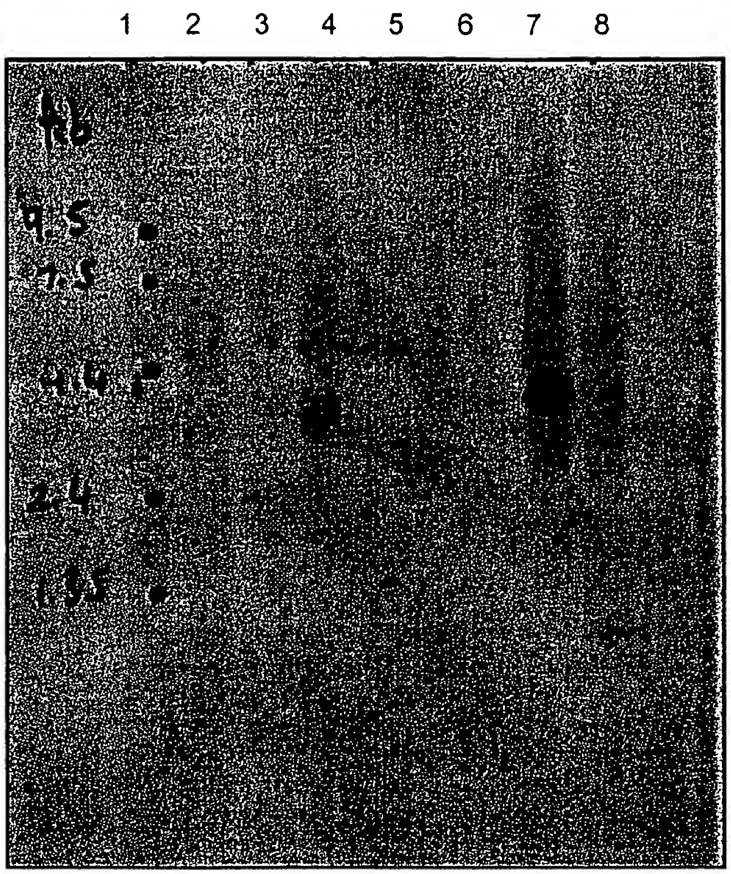
HYBRIDISATION OF NORTHERN BLOT WITH hVR3 PROBE



LANE 1: PERIPHERAL BLOOD LEUKOCYTE
LANE 2: COLON
LANE 3: SMALL INTESTINE
LANE 4: UTERUS
LANE 5: TESTIS
LANE 6: PROSTATE
LANE 7: THYROID
LANE 8: SPLEEN

FIG. 22C

HYBRIDISATION OF A MULTI-TISSUE NORTHERN BLOT WITH THE hVR3 PROBE



LANE 1: HEART
LANE 2: BRAIN
LANE 3: PLACENTA
LANE 4: LUNG
LANE 5: LIVER
LANE 6: SKELETAL MUSCLE
LANE 7: KIDNEY
LANE 8: PANCREAS

SEQUENCE LISTING

<110> Glaxo Group Ltd
Tate, Simon N
Delany, Natalie S
Sanseau, P

<120> Novel Receptors

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ccatcctcat caccgagatc ctccctgaat tcagcccacg acagccaccc cggccgtttt 360

ccttgttctg tgtgggaagg gaggcagcgc ggtggttatc aacctcaccc tgcagaggag 420

gcacctgagg cccagagacg aggagggatg ggtctaaccc agaaccacag atggctctga 480

gccgggggcc tgtccaccct cccaggccga cgtcagtggc cgcaggactg cctgggccct 540

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gttctagggg gctgggggca gcagcaagtt ggagttttgg ggtaccctgc ttcacagggc 660

ccttggcaag gagggcaggt ggggtctaag gacaagcagt ccttactttg ggagtcaacc 720

ccggcgtggt ggctgctgca ggttgcacac tgggccacag aggatccagc aagg atg    777
                                                            Met
                                                              1

aag aaa tgg agc agc aca gac ttg ggg gca gct gcg gac cca ctc caa    825
Lys Lys Trp Ser Ser Thr Asp Leu Gly Ala Ala Ala Asp Pro Leu Gln
            5                  10                  15

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Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg Pro
        20                  25                  30

cct cca gcc aag ccc cag ctc tcc acg gcc aag agc cgc acc cgg ctc    921
Pro Pro Ala Lys Pro Gln Leu Ser Thr Ala Lys Ser Arg Thr Arg Leu
    35                  40                  45

ttt ggg aag ggt gac tcg gag gag gct ttc ccg gtg gat tgc cct cac    969
Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro His
 50                  55                  60                  65
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Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro Val
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Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu Leu
85 90 95

tcc cag gac tct gtc gcc gcc agc acc gag aag acc ctc agg ctc tat 1113
Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu Tyr
100 105 110

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115 120 125

[redacted]

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Ala Leu Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln Gln
550 555 560

atg ggc atc tat gcc gtc atg ata gag aag atg atc ctg aga gac ctg 2505
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565 570 575

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580 585 590

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Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asp Ser Leu Pro Ser
595 600 605

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Glu Ser Thr Ser His Arg Trp Arg Gly Pro Ala Cys Arg Pro Pro Asp
610 615 620 625

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Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe
630 635 640

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645 650 655

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Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile
660 665 670

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675 680 685

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Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr
690 695 700 705

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725 730 735

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740 745 750

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755 760 765

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Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala Ser
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805 810 815

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gaatgtttga aagatcttcc atttctagga accccagtcc tgcttctccg caatggcaca 4284

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35 40 45

Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
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His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
65 70 75 80

Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
85 90 95

Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu
100 105 110

Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
115 120 125

Asp Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
130 135 140

Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu
145 150 155 160

Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
165 170 175

Leu Leu Glu Ile Ala Arg Gln Thr Asp Ser Leu Lys Glu Leu Val Asn
180 185 190

Ala Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile
195 200 205

Ala Ile Glu Arg Arg Asn Met Ala Leu Val Thr Leu Leu Val Glu Asn
210 215 220

Gly Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr
225 230 235 240

Lys Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
245 250 255

Ala Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser
260 265 270

Trp Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val
275 280 285

Leu His Ala Leu Val Glu Val Ala Asp Asn Thr Ala Asp Asn Thr Lys
290 295 300

Phe Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu
305 310 315 320

His Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr
325 330 335

Pro Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr
340 345 350

Ile Leu Gln Arg Glu Ile Gln Glu Pro Glu Cys Arg His Leu Ser Arg
355 360 365

Lys Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp
370 375 380

Leu Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile
385 390 395 400

Ala Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val
405 410 415

Glu Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys
420 425 430

Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile
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Phe Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe
450 455 460

Lys Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
465 470 475 480

Ser Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
485 490 495

Leu Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser
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Glu Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val
515 520 525

Leu Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser
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Leu Ala Leu Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln
545 550 555 560

Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
565 570 575

Leu Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser
580 585 590

Thr Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asp Ser Leu Pro
595 600 605

Ser Glu Ser Thr Ser His Arg Trp Arg Gly Pro Ala Cys Arg Pro Pro
610 615 620

Asp Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys
625 630 635 640

Phe Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe
645 650 655

Lys Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr
660 665 670

Ile Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn
675 680 685

Lys Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile
690 695 700

Thr Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala
705 710 715 720

Phe Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys
725 730 735

Asp Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr
740 745 750

Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu
755 760 765

Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser
770 775 780

Gly Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala
785 790 795 800

Ser Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg
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Pro Ala Ala Ser Gly Glu Lys
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35 40 45

Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Ser Pro L u Asp Cys Pro
50 55 60

Tyr Glu Glu Gly Gly Leu Ala Ser Cys Pro Ile Ile Thr Val Ser Ser
65 70 75 80

Val Leu Thr Ile Gln Arg Pro Gly Asp Gly Pro Ala Ser Val Arg Pro
85 90 95

Ser Ser Gln Asp Ser Val Ser Ala Gly Glu Lys Pro Pro Arg Leu Tyr
100 105 110

Asp Arg Arg Ser Ile Phe Asp Ala Val Ala Gln Ser Asn Cys Gln Glu
115 120 125

Leu Glu Ser Leu Leu Pro Phe Leu Gln Arg Ser Lys Lys Arg Leu Thr
130 135 140

Asp Ser Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu Lys
145 150 155 160

Ala Met Leu Asn Leu His Asn Gly Gln Asn Asp Thr Ile Ala Leu Leu
165 170 175

Leu Asp Val Ala Arg Lys Thr Asp Ser Leu Lys Gln Phe Val Asn Ala
180 185 190

Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile Ala
195 200 205

Ile Glu Arg Arg Asn Met Thr Leu Val Thr Leu Leu Val Glu Asn Gly
210 215 220

Ala Asp Val Gln Ala Ala Ala Asn Gly Asp Phe Phe Lys Lys Thr Lys
225 230 235 240

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Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala
245 250 255

Cys Thr Asn Gln Leu Ala Ile Val Lys Phe Leu Leu Gln Asn Ser Trp
260 265 270

Gln Pro Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val Leu
275 280 285

His Ala Leu Val Glu Val Ala Asp Asn Thr Val Asp Asn Thr Lys Phe
290 295 300

Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu His
305 310 315 320

Pro Thr Leu Lys Leu Glu Glu Ile Thr Asn Arg Lys Gly Leu Thr Pro
325 330 335

Leu Ala Leu Ala Ala Ser Ser Gly Lys Ile Gly Val Leu Ala Tyr Ile
340 345 350

Leu Gln Arg Glu Ile His Glu Pro Glu Cys Arg His Leu Ser Arg Lys
355 360 365

Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp Leu
370 375 380

Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile Ala
385 390 395 400

Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val Glu
405 410 415

Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys Arg
420 425 430

Ile Phe Tyr Phe Asn Phe Phe Val Tyr Cys Leu Tyr Met Ile Ile Phe
435 440 445

Thr Ala Ala Ala Tyr Tyr Arg Pro Val Glu Gly Leu Pro Pro Tyr Lys
450 455 460

Leu Lys Asn Thr Val Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
465 470 475 480

Ser Val Ser Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
485 490 495

Leu Gln Arg Arg Pro Ser Leu Lys Ser Leu Phe Val Asp Ser Tyr Ser
500 505 510

Glu Ile Leu Phe Phe Val Gln Ser Leu Phe Met Leu Val Ser Val Val
515 520 525

Leu Tyr Phe Ser Gln Arg Lys Glu Tyr Val Ala Ser Met Val Phe Ser
530 535 540

Leu Ala Met Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln
545 550 555 560

Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
565 570 575

Leu Cys Arg Phe Met Phe Val Tyr Leu Val Phe Leu Phe Gly Phe Ser
580 585 590

Thr Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asn Ser Leu Pro
595 600 605

Met Glu Ser Thr Pro His Lys Cys Arg Gly Ser Ala Cys Lys Pro Gly
610 615 620

Asn Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe
625 630 635 640

Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe Lys
645 650 655

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Ala Val Phe Il  Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile
            660                 665                 670

Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn Lys
        675                 680                 685

Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr
    690                 695                 700

Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala Phe
705                 710                 715                 720

Arg Ser Gly Lys Leu Leu Gln Val Gly Phe Thr Pro Asp Gly Lys Asp
                725                 730                 735

Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr Trp
            740                 745                 750

Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly
        755                 760                 765

Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Gly Arg Val Ser Gly
    770                 775                 780

Arg Asn Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Asp Ala Ser
785                 790                 795                 800

Thr Arg Asp Arg His Ala Thr Gln Gln Glu Glu Val Gln Leu Lys His
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Tyr Thr Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Asp Ser
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Met Val Pro Gly Glu Lys
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gagtgtgtgc aggccaggga gggctttcca gaggagccca gttgagctgg aacaccagtg 180

gggaggagtt gaccagcaaa ggtgcaggga gggatcagca ctttgcactg gggagcagag 240

tttgtgcact ggggaagtca actcaagtat tggagcctca gtttcctgtt ctgtaaaatg 300

ggttcatcat gacagtgttt gatgaggaaa aggactgccg gcctacacag caagtccaca 360

tggattttct gagcccctcc tgtgcctgaa gcccacggtt aatggttctg ccttagcagg 420

tgcttaccac gtgccaggca ctgcactgca ctggccactg gactgcatgt tctgtccatg 480

aggcttggat atccccatct tacagatcag gaagctgagg ctatgaaatg tcgacttgct 540

caatgtcatg gaatgactaa gtgtggagcc tggatttgaa cttggctctc tggggctcca 600

aagctggctt tcttggtcag cagtagggtc tgggatccaa gtatggggtc ccagcttgac 660

cctgaagtcc accctctttc agcta atg ccc agg gta gtt gga cct ggg gcc   712
                            Met Pro Arg Val Val Gly Pro Gly Ala
                             1               5
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aat ttg tgt ttc cag gtt cgt gaa aga ggc tcc tgt tgc agt tcc cgc 760
Asn Leu Cys Phe Gln Val Arg Glu Arg Gly Ser Cys Cys Ser Ser Arg
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ctg agg ctg gcg gcc aac cac atc tgg gag tgg cct ccc tgt gcc cct 808
Leu Arg Leu Ala Ala Asn His Ile Trp Glu Trp Pro Pro Cys Ala Pro
30 35 40

gtc att aca acg gtg gct ttg aag cag ctg gca gca ctg ctg ctt gtc 856
Val Ile Thr Thr Val Ala Leu Lys Gln Leu Ala Ala Leu Leu Leu Val
45 50 55

cac gtg gga ggg ggc ttc ctg gag ccc ccg ccc ctg gcc ggg ttc tgc 904
His Val Gly Gly Gly Phe Leu Glu Pro Pro Pro Leu Ala Gly Phe Cys
60 65 70

ctg act ccc ctt tca ttc cct tgc agg ctg agc agt gca gac ggg cct 952
Leu Thr Pro Leu Ser Phe Pro Cys Arg Leu Ser Ser Ala Asp Gly Pro
75 80 85

ggg gca ggc atg gcg gat tcc agc gaa ggc ccc cgc gcg ggg ccc ggg 1000
Gly Ala Gly Met Ala Asp Ser Ser Glu Gly Pro Arg Ala Gly Pro Gly
90 95 100 105

gag gtg gct gag ctc ccc ggg gat gag agt ggc acc cca ggt ggg gag 1048
Glu Val Ala Glu Leu Pro Gly Asp Glu Ser Gly Thr Pro Gly Gly Glu
110 115 120

gct ttt cct ctc tcc tcc ctg gcc aat ctg ttt gag ggg gag gat ggc 1096
Ala Phe Pro Leu Ser Ser Leu Ala Asn Leu Phe Glu Gly Glu Asp Gly
125 130 135

tcc ctt tcg ccc tca ccg gct gat gcc agt cgc cct gct ggc cca ggc 1144
Ser Leu Ser Pro Ser Pro Ala Asp Ala Ser Arg Pro Ala Gly Pro Gly
140 145 150

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Gly Val Pro Asn Pro Ile Asp Leu Leu Glu Ser Thr Leu Tyr Glu Ser
170 175 180 185

tcg gtg gtg cct ggg ccc aag aaa gca ccc atg gac tca ctg ttt gac 1288
Ser Val Val Pro Gly Pro Lys Lys Ala Pro Met Asp Ser Leu Phe Asp
190 195 200

tac ggc acc tat cgt cac cac tcc agt gac aac aag agg tgg agg aag 1336
Tyr Gly Thr Tyr Arg His His Ser Ser Asp Asn Lys Arg Trp Arg Lys
205 210 215

aag atc ata gag aag cag ccg cag agc ccc aaa gcc cct gcc cct cag 1384
Lys Ile Ile Glu Lys Gln Pro Gln Ser Pro Lys Ala Pro Ala Pro Gln
220 225 230

ccg ccc ccc atc ctc aaa gtc ttc aac cgg cct atc ctc ttt gac atc 1432
Pro Pro Pro Ile Leu Lys Val Phe Asn Arg Pro Ile Leu Phe Asp Ile
235 240 245

gtg tcc cgg ggc tcc act gct gac ctg gac ggg ctg ctc cca ttc ttg 1480
Val Ser Arg Gly Ser Thr Ala Asp Leu Asp Gly Leu Leu Pro Phe Leu
250 255 260 265

ctg acc cac aag aaa cgc cta act gat gag gag ttt cga gag cca tct 1528
Leu Thr His Lys Lys Arg Leu Thr Asp Glu Glu Phe Arg Glu Pro Ser
270 275 280

acg ggg aag acc tgc ctg ccc aag gcc ttg ctg aac ctg agc aat ggc 1576
Thr Gly Lys Thr Cys Leu Pro Lys Ala Leu Leu Asn Leu Ser Asn Gly
285 290 295

cgc aac gac acc atc cct gtg ctg ctg gac atc gcg gag cgc acc ggc 1624
Arg Asn Asp Thr Il Pro Val Leu Leu Asp Ile Ala Glu Arg Thr Gly
300 305 310

aac atg cgg gag ttc att aac tcg ccc ttc cgt gac atc tac tat cga 1672
Asn Met Arg Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg
315 320 325

ggt cag aca gcc ctg cac atc gcc att gag cgt cgc tgc aaa cac tac 1720
Gly Gln Thr Ala Leu His Ile Ala Ile Glu Arg Arg Cys Lys His Tyr
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Val Glu Leu Leu Val Ala Gln Gly Ala Asp Val His Ala Gln Ala Arg
350 355 360

ggg cgc ttc ttc cag ccc aag gat gag ggg ggc tac ttc tac ttt ggg 1816
Gly Arg Phe Phe Gln Pro Lys Asp Glu Gly Gly Tyr Phe Tyr Phe Gly
365 370 375

gag ctg ccc ctg tcg ctg gct gcc tgc acc aac cag ccc cac att gtc 1864
Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Asn Gln Pro His Ile Val
380 385 390

aac tac ctg acg gag aac ccc cac aag aag gcg gac atg cgg cgc cag 1912
Asn Tyr Leu Thr Glu Asn Pro His Lys Lys Ala Asp Met Arg Arg Gln
395 400 405

gac tcg cga ggc aac aca gtg ctg cat gcg ctg gtg gcc att gct gac 1960
Asp Ser Arg Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp
410 415 420 425

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430 435 440

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ctc aac aac gac ggc ctc tcg ccc ctc atg atg gct gcc aag acg ggc 2104
Leu Asn Asn Asp Gly Leu Ser Pro Leu Met Met Ala Ala Lys Thr Gly
460 465 470

aag att ggg atc ttt cag cac atc atc cgg cgg gag gtg acg gat gag 2152
Lys Ile Gly Ile Phe Gln His Ile Ile Arg Arg Glu Val Thr Asp Glu
475 480 485

gac aca cgg cac ctg tcc cgc aag tcc aag gac tgg gcc tat ggg cca 2200
Asp Thr Arg His Leu Ser Arg Lys Ser Lys Asp Trp Ala Tyr Gly Pro
490 495 500 505

gtg tat tcc tcg ctt tat gac ctc tcc tcc ctg gac acg tgt ggg gaa 2248
Val Tyr Ser Ser Leu Tyr Asp Leu Ser Ser Leu Asp Thr Cys Gly Glu
510 515 520

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Glu Ala Ser Val Leu Glu Ile Leu Val Tyr Asn Ser Lys Ile Glu Asn
525 530 535

cgc cac gag atg ctg gct gtg gag ccc atc aat gaa ctg ctg cgg gac 2344
Arg His Glu Met Leu Ala Val Glu Pro Ile Asn Glu Leu Leu Arg Asp
540 545 550

aag tgg cgg aag ttc ggg gcc gtc tcc ttc tac atc aac gtg gtc tcc 2392
Lys Trp Arg Lys Phe Gly Ala Val Ser Phe Tyr Ile Asn Val Val Ser
555 560 565

tac ctg tgt gcc atg gtt atc ttc act ctc acc gcc tac tac cag ccg 2440
Tyr Leu Cys Ala Met Val Ile Phe Thr Leu Thr Ala Tyr Tyr Gln Pro
570 575 580 585

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Leu Glu Gly Thr Pro Pro Tyr Pro Tyr Arg Thr Thr Val Asp Tyr Leu
                590                 595                 600

cgg ctg gct ggc gag gtc att acg ctc ttc act ggg gtc ctg ttc ttc    2536
Arg Leu Ala Gly Glu Val Ile Thr Leu Phe Thr Gly Val Leu Phe Phe
            605                 610                 615

ttc acc aac atc aaa gac ttg ttc atg aag aaa tgc cct gga gtg aat    2584
Phe Thr Asn Ile Lys Asp Leu Phe Met Lys Lys Cys Pro Gly Val Asn
        620                 625                 630

tct ctc ttc att gat ggc tcc ttc cag ctg ctc tac ttc atc tac tct    2632
Ser Leu Phe Ile Asp Gly Ser Phe Gln Leu Leu Tyr Phe Ile Tyr Ser
    635                 640                 645

gtc ctg gtg atc gtc tca gca gcc ctc tac ctg gca ggg atc gag gcc    2680
Val Leu Val Ile Val Ser Ala Ala Leu Tyr Leu Ala Gly Ile Glu Ala
650                 655                 660                 665

tac ctg gcc atg atg gtc ttt gcc ctg gtc ctg ggc tgg atg aat gcc    2728
Tyr Leu Ala Met Met Val Phe Ala Leu Val Leu Gly Trp Met Asn Ala
                670                 675                 680

ctt tac ttc acc cgt ggg ctg aag ctg acg ggg acc tat agc atc atg    2776
Leu Tyr Phe Thr Arg Gly Leu Lys Leu Thr Gly Thr Tyr Ser Ile Met
            685                 690                 695

atc cag aag att ctc ttc aag gac ctt ttc cga ttc ctg ctc gtc tac    2824
Ile Gln Lys Ile Leu Phe Lys Asp Leu Phe Arg Phe Leu Leu Val Tyr
        700                 705                 710

ttg ctc ttc atg atc ggc tac gct tca gcc ctg gtc tcc ctc ctg aac    2872
Leu Leu Phe Met Ile Gly Tyr Ala Ser Ala Leu Val Ser Leu Leu Asn
    715                 720                 725
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730 735 740 745

gtg ccc act tac ccc tcg tgc cgt gac agc gag acc ttc agc acc ttc 2968
Val Pro Thr Tyr Pro Ser Cys Arg Asp Ser Glu Thr Phe Ser Thr Phe
750 755 760

ctc ctg gac ctg ttt aag ctg acc atc ggc atg ggc gac ctg gag atg 3016
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765 770 775

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780 785 790

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Tyr Ile Ile Leu Thr Ser Val Leu Leu Leu Asn Met Leu Ile Ala Leu
795 800 805

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Met Gly Glu Thr Val Gly Gln Val Ser Lys Glu Ser Lys His Ile Trp
810 815 820 825

aag ctg cag tgg gcc acc acc atc ctg gac att gag cgc tcc ttc ccc 3208
Lys Leu Gln Trp Ala Thr Thr Ile Leu Asp Ile Glu Arg Ser Phe Pro
830 835 840

gta ttc ctg agg aag gcc ttc cgc tct ggg gag atg gtc acc gtg ggc 3256
Val Phe Leu Arg Lys Ala Phe Arg Ser Gly Glu Met Val Thr Val Gly
845 850 855

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Lys Ser Ser Asp Gly Thr Pro Asp Arg Arg Trp Cys Phe Arg Val Asp
860 865 870

gag gtg aac tgg tct cac tgg aac cag aac ttg ggc atc atc aac gag 3352
Glu Val Asn Trp Ser His Trp Asn Gln Asn Leu Gly Ile Ile Asn Glu
875 880 885

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Asp Pro Gly Lys Asn Glu Thr Tyr Gln Tyr Tyr Gly Phe Ser His Thr
890 895 900 905

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Val Gly Arg Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg Val
910 915 920

gtg gaa ctg aac aag aac tcg aac ccg gac gag gtg gtg gtg cct ctg 3496
Val Glu Leu Asn Lys Asn Ser Asn Pro Asp Glu Val Val Val Pro Leu
925 930 935

gac agc atg ggg aac ccc cgc tgc gat ggc cac cag cag ggt tac ccc 3544
Asp Ser Met Gly Asn Pro Arg Cys Asp Gly His Gln Gln Gly Tyr Pro
940 945 950

cgc aag tgg agg act gat gac gcc ccg ctc tag ggactgcagc ccagccccag 3597
Arg Lys Trp Arg Thr Asp Asp Ala Pro Leu
955 960

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35 40 45

Lys Gln Leu Ala Ala Leu Leu Leu Val His Val Gly Gly Gly Phe Leu
50 55 60

Glu Pro Pro Pro Leu Ala Gly Phe Cys Leu Thr Pro Leu Ser Phe Pro
65 70 75 80

Cys Arg Leu Ser Ser Ala Asp Gly Pro Gly Ala Gly Met Ala Asp Ser
85 90 95

Ser Glu Gly Pro Arg Ala Gly Pro Gly Glu Val Ala Glu Leu Pro Gly
100 105 110

Asp Glu Ser Gly Thr Pro Gly Gly Glu Ala Phe Pro Leu Ser Ser Leu
115 120 125

Ala Asn Leu Phe Glu Gly Glu Asp Gly Ser Leu Ser Pro Ser Pro Ala
130 135 140

Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg Pro Asn Leu Arg
145 150 155 160

Met Lys Phe Gln Gly Ala Phe Arg Lys Gly Val Pro Asn Pro Ile Asp
165 170 175

Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val Pro Gly Pro Lys
180 185 190

Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr Tyr Arg His His
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Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro Ile Leu Lys Val
225 230 235 240

Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg Gly Ser Thr Ala
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Asp Leu Asp Gly Leu Leu Pro Phe Leu Leu Thr His Lys Lys Arg Leu
260 265 270

Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys Thr Cys Leu Pro
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Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg Glu Phe Ile Asn
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Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr Ala Leu His Ile
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340 345 350

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Asp Glu Gly Gly Tyr Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
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His Lys Lys Ala Asp Met Arg Arg Gln Asp Ser Arg Gly Asn Thr Val
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Glu Pro Ile Asn Glu Leu Leu Arg Asp Lys Trp Arg Lys Phe Gly Ala
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[redacted]

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Thr Leu Phe Thr Gly Val Leu Phe Phe Phe Thr Asn Ile Lys Asp Leu
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Phe Gln Leu Leu Tyr Phe Ile Tyr Ser Val Leu Val Ile Val Ser Ala
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Ala Leu Tyr Leu Ala Gly Ile Glu Ala Tyr Leu Ala Met Met Val Phe
660 665 670

Ala Leu Val Leu Gly Trp Met Asn Ala Leu Tyr Phe Thr Arg Gly Leu
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Lys Leu Thr Gly Thr Tyr Ser Ile Met Ile Gln Lys Ile Leu Phe Lys
690 695 700

Asp Leu Phe Arg Phe Leu Leu Val Tyr Leu Leu Phe Met Ile Gly Tyr
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Ala Ser Ala Leu Val Ser Leu Leu Asn Pro Cys Ala Asn Met Lys Val
725 730 735

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740 745 750

Arg Asp Ser Glu Thr Phe Ser Thr Phe Leu Leu Asp Leu Phe Lys Leu
755 760 765

Thr Ile Gly Met Gly Asp Leu Glu Met Leu Ser Ser Thr Lys Tyr Pro
770 775 780

Val Val Phe Ile Ile Leu Leu Val Thr Tyr Ile Ile Leu Thr Ser Val
785 790 795 800

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Asn Gln Asn Leu Gly Ile Ile Asn Glu Asp Pro Gly Lys Asn Glu Thr
885 890 895

Tyr Gln Tyr Tyr Gly Phe Ser His Thr Val Gly Arg Leu Arg Arg Asp
900 905 910

Arg Trp Ser Ser Val Val Pro Arg Val Val Glu Leu Asn Lys Asn Ser
915 920 925

Asn Pro Asp Glu Val Val Val Pro Leu Asp Ser Met Gly Asn Pro Arg
930 935 940

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<211> 764

<212> PRT

<213> Homo sapiens

<400> 6

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50 55 60

Gly Ala Ser Gln Pro Asp Pro Asn Arg Phe Asp Arg Asp Arg Leu Phe
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Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
85 90 95

Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
100 105 110

Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys
115 120 125

Asp Gly Val Asn Ala Cys Ile Leu Pro Leu Leu Gln Ile Asp Arg Asp
130 135 140

Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
145 150 155 160

Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu
165 170 175

Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg
180 185 190

Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe
195 200 205

Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val
210 215 220

Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala
225 230 235 240

Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser
245 250 255

Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly
260 265 270

Leu Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp
275 280 285

Ile Arg Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu
290 295 300

Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly
305 310 315 320

Leu Ser His Leu Ser Arg Lys Phe Thr Glu Trp Cys Tyr Gly Pro Val
325 330 335

Arg Val Ser Leu Tyr Asp Leu Ala Ser Val Asp Ser Cys Glu Glu Asn
340 345 350

Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His
355 360 365

Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp
370 375 380

Asp Leu Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile
385 390 395 400

Tyr Met Phe Ile Ph Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys
405 410 415

Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu
420 425 430

Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val
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Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu
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Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln
515 520 525

Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Ile Tyr Leu Val
530 535 540

Phe Leu Phe Gly Phe Ala Val Ala Leu Val Ser Leu Ser Gln Glu Ala
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565 570 575

Pro Met Glu Gly Gln Glu Asp Glu Gly Asn Gly Ala Gln Tyr Arg Gly
580 585 590

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Glu Leu Ala Phe Gln Glu Gln Leu His Phe Arg Gly Met Val Leu Leu
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Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Ile Leu Leu Leu Asn Met
625                 630                 635                 640

Leu Ile Ala Leu Met Ser Glu Thr Val Asn Ser Val Ala Thr Asp Ser
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Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met Glu
            660                 665                 670

Asn Gly Tyr Trp Trp Cys Arg Lys Lys Gln Arg Ala Gly Val Met Leu
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Thr Val Gly Thr Lys Pro Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe
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Arg Val Glu Glu Val Asn Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr
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Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn
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Pro Val Leu Ala Ser Pro Pro Lys Glu Asp Glu Asp Gly Ala Ser Glu
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<223> Description of Artificial Sequence: Primer

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<210> 40

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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/09284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12N15/85 C12N5/10 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CATERINA, M.J. ET AL.: "The capsaicin receptor: a heat-actvated ion channel in the pain pathway" NATURE, vol. 389, no. 6653, 23 October 1997 (1997-10-23), pages 816-824, XP002075020 cited in the application abstract page 819; figures 5A,C page 820, column 2, line 13 -page 821, column 1, line 29 page 823, column 2, line 13 - line 14	1-3,6,9, 14-16, 45-47
X	page 817, column 2, line 12 -page 820, column 1, line 21	26
A	page 823, column 2, line 19 -page 824, column 1, line 5	4,5,7,8, 10-13, 17-25, 48-51

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 April 2000	09/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
	page 817; figures 2A-C page 818; figures 3A-F	
X	EMBL Database, Heidelberg, FRG Emest2 accession number AA700891 22 December 1997 Hillier, L. ET AL.: "zj40d01.s1 Soares fetal liver spleen 1NFLS S1 Homo sapiens cDNA clone 452737 3'" XP002135284 the whole document	6,7
X	EMBL Database, Heidelberg, FRG Emest6 accession number AI089668 19 August 1998 NCI/NINDS-CGAP: "qa10f06.x1 NCI_CGAP_Brn23 Homo sapiens cDNA clone IMAGE:1686371 3'" XP002135285 the whole document	6,8
X	BIRO, T. ET AL.: "Recent Advances in Understanding of Vanilloid Receptors: A Therapeutic Target for Treatment of Pain and Inflammation in Skin" JOURNAL OF INVESTIGATIVE DERMATOLOGY SYMPOSIUM PROCEEDINGS, vol. 2, no. 1, August 1997 (1997-08), pages 56-60, XP002075021	48,49
A	abstract page 57; table 1 page 58, column 1, line 8 -column 2, line 16	50,51
P,X	WO 99 37675 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 29 July 1999 (1999-07-29) abstract page 1, line 1 -page 3, line 30 SEQ ID NOS: 33 and 34 page 100 -page 106 page 58 -page 59; claims 1,24-6,8-14,19	1,2,4,6, 7,9,10, 12,14, 15,23, 24,26, 45,46, 48,50

-/--

INTERNATIONAL SEARCH REPORT

Inte..tional Application No
PCT/EP 99/09284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP 0 943 683 A (SMITHKLINE BEECHAM PLC) 22 September 1999 (1999-09-22) abstract page 2, line 1 - line 31 SEQ ID NOS: 1 and 2 page 14-16 page 36 -page 37; claims 1-14 -----	1,2,4,6, 7,9,10, 12,14, 15,23, 24,26, 45,46

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT	International application No. PCT/EP 99/09284

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 27-45
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 99/09284

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 27-45

Claims 27 - 45 refer to a compound which modulates human vanilloid receptor activity without giving a true technical characterization. Moreover, except two compounds already known in the prior art, no such compounds are defined in the application. In consequence, the scopes of said claims are ambigous and vague, and their subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).

No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/09284

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9937675	A	29-07-1999	AU	2466799	A	09-08-1999
			AU	9115698	A	08-03-1999
			WO	9909140	A	25-02-1999
EP 0943683	A	22-09-1999	JP	11279196	A	12-10-1999